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Therapeutic Efficacy Of Gold Nanoparticles And Thyme Extracts Against Ticks In Vivo

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Abstract: This study aims to explore the therapeutic efficacy of gold nanoparticles (AuNPs) and thyme Thymus vulgaris extracts against ticks, to examine the effectiveness of gold nanoparticles and thyme extract as anti-tick agents. And the study involved collecting 268 tick samples from cattle in various areas of Salah al-Din Governorate, Iraq.

Gold nanoparticles were prepared using the citrate reduction method, with the particles having a diameter of approximately 20 nanometers. The effect of different concentrations of gold nanoparticles (2, 3, 4, and 5 mg/ml) and thyme extract (12.5, 25, 50, and 100 mg/ml) on ticks was tested, with their effects monitored over different time periods (from 30 minutes to 24 hours). The results showed that both gold nanoparticles and thyme extract exhibited lethal activity against ticks, with efficacy increasing with increasing concentration and exposure periods.

The synergistic effect between gold nanoparticles and thyme extract was also evaluated, and the results showed that this combination increased the effectiveness of the treatment compared to using either extract alone. This combination of the two extracts improved the speed of action and increased the kill rate.

The main conclusions of this study are that gold nanoparticles and thyme extract can be effective alternatives to traditional chemical pesticides for tick control. The synergistic interaction between them provides a stronger effect, suggesting the potential for using this combination in tick control strategies in an environmentally friendly and more sustainable manner.

Keyword: Therapeutic efficacy, gold nanoparticles, thyme extracts, ticks, In Vivo.

Introduction: Ticks are small to medium-sized, blood-feeding ectoparasites. They belong to the phylum Arthropoda, class Arachnida, within the order Ixodida, which includes three main families: the hard tick family (Ixodidae), the soft tick family (Argasidae), and a rare family Nuttalliellidae, found in Africa (Soulsby, 1982).

Ticks transmit numerous pathogens to vertebrates, including viruses, bacteria, protozoa, and helminths. These pathogens are believed to be responsible for more than 100,000 cases of human disease worldwide. Ticks are the second most common vector of human diseases after mosquitoes and are also the primary source of transmission of diseases affecting domestic and wild animals (Estrada-Peñ et al., 2008). Ticks are vectors of many diseases in humans and animals, directly impacting animal health and the veterinary economy, as they transmit numerous protozoan parasites that lead to deteriorating animal health and reduced productivity (Capinera, 2020).

With advances in nanotechnology and medical science, numerous nanoparticles and nanomaterials derived from various elements such as gold, silver, iron, copper, cobalt, and platinum have been developed, which are manufactured using biological, physical, or chemical methods. The ability to control the properties of nanoparticles, including their physical, chemical, and biological properties, opens up vast opportunities to explore their applications in drug delivery. Among organic and inorganic nanoparticles, gold is a versatile material that has been used in medical applications for centuries, thanks to its antibacterial properties, corrosion resistance, and oxidation resistance (Al-Dulimi, 2021). Gold nanoparticles are among the most important materials in the fields of medicine and pharmacology, where they are widely used in diagnostic and therapeutic applications. Nanogold possesses unique properties due to its small size and high surface density, making it suitable for use in drug delivery and targeted therapy. These particles are also used in the diagnosis of diseases through imaging techniques such as magnetic resonance imaging (MRI) and nuclear medicine. They can also be used in the treatment of tumors and the delivery of chemotherapy more precisely and effectively (Castillo-Henríquez et al., 2020).

Medicinal plants play a fundamental role in enhancing human culture as a source of healing and have always been at the forefront of all cultures and civilizations throughout the ages. For thousands of years, medicinal plants have been used to treat health disorders and prevent epidemic diseases (Dar et al., 2017).

Thymus vulgaris L. is a perennial shrub native to the Mediterranean region and cultivated worldwide (Madathil et al., 2022; Premrov Bajuk et al., 2022). It is an aromatic, evergreen, perennial herb with a pleasant aroma. It grows between 20 and 40 cm tall and has square, woody stems covered with brown hairs with several branching at the base. The leaves are small, lanceolate, hairy, and fragrant. The flowers are edible and come in crimson, purple, or white colors. They are bisexual and fragrant (Hammoudi et al., 2022). Thyme (Thymus vulgaris) belongs to the Lamiaceae family of the order Lamiales, which includes 136 species distributed across 29 genera, including Origanum, Thymus, Thymbra spicata, and Satureja thymbra syriacum (Khoury et al., 2016) (Flores et al., 2018).

Thyme is a plant rich in bioactive compounds that give it a wide range of medicinal properties. Studies have demonstrated its therapeutic effects, demonstrating its ability to combat oxidation, inflammation, and microbiology (Vassiliou et al., 2023). The plant's chemical components, such as thymol, carvacrol, and various acids, contribute to its pharmacological potential, including its antibacterial and antifungal properties (Rodrigues et al., 2022).

The study aimed to investigate the effect of gold nanoparticles on the treatment of ticks in vitro, while also investigating the effect of an aqueous extract of thyme on the treatment of ticks in vitro.

Materials and Methods:

1- Gold nanoparticles (GNPs): Gold nanoparticles (GNPs) were prepared chemically. Citrate-coated gold nanoparticles (GNPs) with a diameter of 20 nm were prepared by reducing 1 mmol of $HAuCl_4$ with sodium citrate (1%) according to the method (Herizchi et al., 2016; Leng et al., 2015).

2- Preparation of thyme aqueous extract: Thyme plants were obtained from local markets, washed and cleaned of dirt and dust, and dried in an oven at room temperature until dry to prevent leaf rot and fungal infection. The dried flowers and leaves were stored in tightly sealed containers under moisture-free conditions until used in the study. It was added to warm water at 40°C until the powder dissolved. The solution was then filtered, the filtrate was collected, and the precipitate was left. The plant suspension was placed on a rotary evaporator for two hours, after which it was filtered through four pieces of gauze (Gasior et al., 1999).

3. Tick Sample Collection Area: 268 samples were collected from 60 cows. The collection process took place in several areas in the Yathrib district (Al-Ajiliyyah, Al-Jam'iyah, Al-Faris, Al-Gharir, Al-Bu Hassan, Al-Aliya District, Al-Gharbiyah, and Al-Zour) in Salah al-Din Governorate, during the period from September 2024 to March 2025. The samples were placed in sterile plastic bottles containing 70% ethyl alcohol with tight caps. 4-8 Effect of gold nanoparticle extract on ticks

To study the effect of gold nanoparticle extract on tick samples, tick samples were distributed into three replicates of each concentration, with 10 ticks per replicate. Four concentrations (2, 3, 4, and 5) mg/ml were used for each extract, in addition to distilled water (mg/ml) (negative control group) and the tick control drug deltametrine at a concentration of (0.001) mg/ml as a positive control group.

3-10 Effect of thyme aqueous extract on ticks

The study examined the effect of thyme aqueous extract on tick samples. Tick samples were distributed into three replicates of each concentration, with 10 ticks per replicate. Four concentrations were used (100, 50%, 25%, and 12.5 mg/ml) of each aqueous extract, in addition to 0 mg/ml distilled water (negative control group) and 0.001 mg/ml of deltametrine, the anti-tick drug, as a positive control group.

Tick samples were immersed in each concentration for 10 minutes in a Petri dish, They were then transferred to another Petri dish and observed, with observations recorded for each replicate at varying time intervals (30 minutes, 1 hour, 3 hours, 6 hours, 12 hours, and 24 hours). Activity, behavior, and mortality were monitored, and the percentage of dead ticks was calculated for each concentration according to the time of exposure to the extract.

Results and Discussion: It was found that the classified species belong to the genus Hyalomma, which belongs to hard ticks. The results of the current study are consistent with what Hasson (2016) recorded regarding the prevalence of two types of ticks. Hyalomma anatolicum and H. anatolicum excavatum.

These results are consistent with those reported by Shanan et al. (2017), who found that species belonging to Hyalomma were more dominant than species belonging to Rhipicephalus in 12 Iraqi governorates, including Babil, Kirkuk, Diyala, Erbil, Baghdad, Wasit, Maysan, Najaf, Karbala, Muthanna, Dhi Qar, and Basra. The study also agrees with the findings of AbdulKarim and Hatem (2023), who confirmed that the dominant species within the genus Hyalomma in goats examined in Basra Governorate was H. anatolicum.

In a comprehensive review conducted by AbdulKarim et al. (2023) of research conducted in various regions of Iraq, it was found that the genus Hyalomma is the most widespread compared to other tick genera, as it was found in most areas of the country with high infection rates and wide diversity, indicating its ability to adapt to different environmental conditions. The dominance and prevalence of these species are attributed to The suitability of environmental and climatic conditions to their needs, as well as their high adaptability, which explains their prevalence in the Middle East and Africa, is further enhanced by their great diversity compared to other species.

Table (1) shows the results for the number of dead ticks for each concentration, as well as the antiparasitic efficacy of the aqueous extract of thyme at graded concentrations (12.5, 25, 50, and 100 mg/ml) and tick exposure periods of half an hour, 1 hour, 3 hours, 6 hours, 12 hours, and 24 hours. The results showed that the aqueous extract had a lethal effect on ticks comparable to the traditional acaricide (Cyperomethrin), with activity increasing with increasing exposure time and concentration. From Table (1), we note that half an hour after tick exposure to the aqueous extract of thyme at concentrations (100 and 50 mg/ml) had a 0% tick-killing effect compared to the positive control (0%) and the negative control (0%). However, after 24 hours of exposure, the aqueous extract had a 0% tick-killing effect. For the aqueous extract, the concentration (100 mg/ml) showed a significant effect in killing ticks by (66.67%) compared to the positive control (16.67%) and the negative control (0%).

In the current study, it was observed that the aqueous extract of thyme had lethal effects on ticks, comparable to or superior to the insecticide deltamethrin. The results showed that different concentrations of the extract (100, 50, 25, and 12.5 mg/ml) resulted in mortality rates of 66.67%, 50%, 33.33%, and 16.67%, respectively, 24 hours after exposure to the extract, compared to a mortality rate of 16.67% in the positive control group.

The results also demonstrated a direct relationship between increasing the concentration of the aqueous extract of thyme and the duration of tick exposure, on the one hand, and an increased mortality rate, on the other. These results are consistent with previous studies, which have demonstrated that some plants have medicinal properties, including anti-tick activity, as indicated by several previous studies (Silva et al., 2020; Vongkhamchanh et al., 2023).

Thyme is one of the most widely studied medicinal plants worldwide, with its use documented in the treatment of a range of diseases affecting both humans and animals (Veers and Venkatachalam, 2019). According to the researcher's vision, this study may be the first in Iraq to investigate the use of thyme aqueous extract as an anti-tick, due to the lack of previous published studies in this field within the local context.

Table (2) shows the results for the number of dead ticks for each concentration, as well as the antiparasitic efficacy of the gold nanoparticle extract at graded concentrations (2, 3, 4, and 5) ml, and the time of tick exposure to the gold nanoparticle extract. The anti-tick-cidal effects are relatively comparable to the traditional acaricide (delta meterine), and the activity increases with increasing exposure time and concentration. From Table (2), we note that after three hours of tick exposure to the gold nanoparticle extract, the concentration of 5 ml showed a tick-killing effect of 3.33%, while at the same time (6 and 12 hours), the concentration showed a 3.33% effect. After 24 hours, the 2 ml concentration showed a 16.67% tick-killing effect, while the 3.4 ml concentration showed a 3.33% tick-killing effect. The 5 ml concentration showed a 6.67% tick-killing effect, compared to the positive control (16.67%) and the negative control (0%). Table 4-4 shows the relationship between tick mortality and the concentration of the gold nanoparticle extract. Table 3 shows the results of the synergistic effect of gold nanoparticles and the aqueous extract of thyme, showing the number of dead ticks for each concentration, as well as the antiparasitic biological activity, at graded concentrations (2, 3, 4, 5) ml and the duration of tick exposure to the gold nanoparticle extract and thyme extract (half an hour, 3 hours, 6 hours, 12 hours, 24 hours). The results showed that the two extracts had effects relatively comparable to the traditional acaricide deltametrine, with activity increasing with increasing exposure time and concentration.

From Table (3), we note that after 6 hours of tick exposure to the two extracts, concentrations (2, 3, and 5) ml showed a percentage of (16.67%), and concentration (3) ml showed a percentage of (10%). After 12 hours, concentration (2) ml showed a percentage of (20%), while concentrations (3, 4, and 5) ml showed a percentage of (33.33%). After 24 hours, concentration (2) ml showed a percentage of (33.33%), concentration (3) ml showed a percentage of (16.67%), concentration (4) ml showed a percentage of (50%), and concentration (5) ml showed a percentage of (33.33%), compared to the positive control (16.67%) and the negative control (0%).

The synergistic activity of thyme extract and gold nanoparticles demonstrated a high efficiency in eliminating ticks compared to the effect of each of them alone. The mortality rate at the 2 ml concentration reached 33.33%, while at the 3 ml concentration it reached 16.67%. At the 4 ml concentration, the rate increased to 50%, while at the 5 ml concentration it reached 33.33%. Compared to the positive control, which showed a mortality rate of 16.67%, it is clear that the combination of thyme extract and gold nanoparticles significantly enhanced the anti-tick efficacy, especially at the 4 ml concentration.

These results confirm a positive synergistic effect between the two extracts, as the combined treatment contributed to increasing mortality rates and reducing the time lag required for effect. This is consistent with previous studies on the effectiveness of combining plant and mineral compounds in controlling parasites and microorganisms.

Table (1) Effect of aqueous extract of thyme leaves at different concentrations on the number and percentage of dead ticks

Examination duration	drug used	concentration	number of ticks used	number of dead ticks	Percentage %
½ hour	aqueous extract of thyme	100	30	0	**0**
		50	30	0	**0**
		25	30	0	**0**
		12.5	30	0	**0**
	_ conrol	%0	30	0	**0**
	+ control	0.001	30	0	**0**
1 hour	aqueous extract of thyme	100	30	0	**0**
		50	30	0	**0**
		25	30	0	**0**
		12.5	30	0	**0**
	_ conrol	0	30	0	**0**
	+ control	0.001	30	0	**0**
3 hours	aqueous extract of thyme	100	30	0	**0**
		50	30	3	**10**
		25	30	0	**0**
		12.5	30	4	**13.33**
	_ conrol	%0	30	0	**0**
	+ control	0.001	30	0	**0**
6 hours	aqueous extract of thyme	100	30	0	**0**
		50	30	3	**10**
		25	30	2	**%6.67**
		12.5	30	2	**%6.67**
	_ conrol	0	30	0	**0**
	+ control	0.001	30	0	**0**
12 hours	aqueous extract of thyme	100	30	0	**0**
		50	30	3	**0**
		25	30	2	**%6.67**

		12.5	30	2	**%6.67**
	_ conrol	0	30	0	**0**
	+ control	0.001	30	0	**0**
24 hours	aqueous extract of thyme	100	30	20	**%66.67**
		50	30	5	**%16.67**
		25	30	15	**50**
		12.5	30	10	**33.33**
	_ conrol	0	30	0	**0**
	+ control	0.001	30	5	**%16.67**

Table (2) Effect of gold nanoparticles at different concentrations on the number and percentage of dead ticks

Examination duration	**drug used**	**concentration**	**number of ticks used**	**number of dead ticks**	**Percentage %**
½ hour	aqueous extract of thyme	2مل	30	0	**%0**
		3 مل	30	0	**%0**
		4مل	30	0	**%0**
		5مل	30	0	**%0**
	_ conrol	0	30	0	**%0**
	+ control	0.001	30	0	**%0**
1 hour	aqueous extract of thyme	2مل	30	0	**%0**
		3 مل	30	0	**%0**
		4مل	30	0	**%0**
		5مل	30	0	**%0**
	_ conrol	0	30	0	**%0**
	+ control	0.001	30	0	**%0**
3 hours	aqueous extract of thyme	2مل	30	0	**%0**
		3 مل	30	0	**%0**
		4مل	30	0	**%0**

		5مل	30	1	**%3.33**
	_ conrol	0	30	0	**%0**
	+ control	0.001	30	0	**%0**
6 hours	aqueous extract of thyme	2مل	30	0	**%0**
		3 مل	30	0	**%0**
		4مل	30	0	**%0**
		5مل	30	1	**%3.33**
	_ conrol	0	30	0	**%0**
	+ control	0.001	30	0	**%0**
12 hours	aqueous extract of thyme	2مل	30	0	**%0**
		3 مل	30	0	**%0**
		4مل	30	0	**%0**
		5مل	30	1	**%3.33**
	_ conrol	0	30	0	**%0**
	+ control	0.001	30	0	**%0**
24 hours	aqueous extract of thyme	2مل	30	5	**%16.67**
		3 مل	30	1	**%3.33**
		4مل	30	1	**%3.33**
		5مل	30	2	**%6.67**
	_ conrol	0	30	0	**%0**
	+ control	0.001	30	5	**%16.67**

Table (3) synergistic effect of aqueous extract of thyme leaves and gold nanoparticles on the number and percentage of dead ticks

Examination duration	**drug used**	**concentration**	**number of ticks used**	**number of dead ticks**	**Percentage %**
½ hour	aqueous extract of thyme	2مل	30	0	**%0**
		3 مل	30	0	**%0**
		4مل	30	0	**%0**

		5مل	30	0	**%0**
	_ conrol	0	30	0	**%0**
	+ control	0.001	30	0	**%0**
1 hour	aqueous extract of thyme	2مل	30	0	**%0**
		3 مل	30	0	**%0**
		4مل	30	0	**%0**
		5مل	30	0	**%0**
	_ conrol	0	30	0	**%0**
	+ control	0.001	30	0	**%0**
3 hours	aqueous extract of thyme	2مل	30	0	**%0**
		3 مل	30	0	**%0**
		4مل	30	0	**%0**
		5مل	30	0	**%0**
	_ conrol	0	30	0	**%0**
	+ control	0.001	30	0	**%0**
6 hours	aqueous extract of thyme	2مل	30	5	**%16.67**
		3 مل	30	3	**%10**
		4مل	30	5	**%16.67**
		5مل	30	5	**%16.67**
	_ conrol	0	30	0	**%0**
	+ control	0.001	30	0	**%0**
12 hours	aqueous extract of thyme	2مل	30	6	**%20**
		3 مل	30	10	**%33.33**
		4مل	30	10	**%33.33**
		5مل	30	10	**%33.33**
	_ conrol	0	30	0	**%0**
	+ control	0.001	30	0	**%0**
24 hours		2مل	30	10	**%33.33**
		3 مل	30	5	**%16.67**

	aqueous extract of thyme	4مل	30	15	**%50**
		5مل	30	10	**%33.33**
	_ conrol	0	30	0	**%0**
	+ control	0.001	30	5	**%16.67**

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