

Therapeutic Efficacy of Gold Nanoparticles and Thyme Extracts Against Ticks in Vivo

Nabaa Othman Obaid Al-Hashmawi ^{1,a)} and Ashraf Jamal Mahmoud Zangana ^{1,b)}

¹ Tikrit University, College of Education for women, Biology, Iraq.

^{a)} corresponding author: naba.mezher19@st.tu.edu.iq

^{b)} dr.ashraf_bio@tu.edu.iq

Abstract. In the present study, the authors intend to examine the potential of gold nanoparticles and thyme extract as an anti-tick to explore the therapeutic efficacy of the two extracts in the treatment of ticks, gold nanoparticles (AuNPs) and thyme *Thymus vulgaris* extracts. And the research was conducted on 268 tick samples of cattle in different locations of Salah al-Din Governorate, Iraq. The citrate reduction method has been used to prepare gold nanoparticles and the diameter of the particle is about 20 nanometers. The activity of the various 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 experimented and their effects were observed at various time intervals (ranging between 30 minutes and 24 hours). The findings indicated the presence of lethal activity of both gold nanoparticles and thyme extract on ticks, and the effectiveness of both extracts augmented with growing levels of concentration and exposure duration. The synergetic performance of gold nanoparticles and thyme extract was also tested and the findings indicated that this combination enhanced the performance of the treatment as compared to the performance of the extracts used separately. This two extracts combination enhanced the rate of action and kill rate. The principal findings of the given research are that gold nanoparticles and thyme extract may serve as the alternative to conventional chemical pesticides to eliminate ticks. Their synergistic interaction gives them a more potent effect and it is possible to suggest that such a combination can be used in the tick control methods in a more sustainable and environmentally-friendly way.

Keywords: 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 [1]. 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 [2]. 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 [3]. 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. Being able to manage the properties of nanoparticles such as their physical, chemical and biological property presents very many opportunities to explore their usage in drug delivery. Gold is one of the most common organic and inorganic nanoparticles which have been employed in medical practice centuries ago due to its antibacterial properties, resistance to corrosion, and resistance to oxidation [4]. One of the most significant materials used in medicine and pharmacology is gold nanoparticles, as they are commonly used in diagnostic and therapeutic treatment. Nanogold is a product with special characteristics as it is small in size and has high surface density, so it can be utilized in drug delivery and target therapy. The particles are also employed in the diagnosis of diseases in the imaging process like in the magnetic resonance imaging (MRI) and nuclear medicine. They are also applicable in the treatment of tumors and more precise and effective delivery of chemotherapy [5]. Medicinal plants are very essential in boosting the human culture as a source of healing which has always been the leading activity in all cultures and civilizations over the ages. Medicinal plants have been practicing in treating health-related disorders as well as preventing epidemic illnesses; the practice has taken thousands of years [6].

Thymus vulgaris L. is a shrub that is perennial in origin and grows all over the world, although it is native to the Mediterranean region [7]. It is a pleasantly-smelling evergreen, aromatic and perennial herb. It is 20-40 cm

tall with four sided, woody stems and several branching in the base with brown hairs. Its leaves are fragrant, hairy, lanceolate, and small. The flowers are also edible and they have either crimson, purple or white color. They are outdoor and sweet-smelling [8]. Thyme (*Thymus vulgaris*) is a biological agent that contains many bioactive compounds and, therefore, has a wide range of medical applications [9]. Thyme is a plant of the family Lamiaceae, as well as the order Lamiales, containing 136 species belonging to the genera *Origanum*, *Thymus*, *Thymbra spicata*, and *Satureja thymbra syriacum*. Its therapeutic pharmacological opportunities such as antibacterial and antifungal functions are explained by the chemical composition of the plant, which also contains thymol, carvacrol, and other kinds of acids [10].

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*.

EXPERIMENTAL PART

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 HAuCl₄ with sodium citrate (1%) according to the method [11][12].

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 [13].

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 deltamethrin at a concentration of (0.001) mg/ml as a positive control group. 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 deltamethrin, 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.

RESULT 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) [14] 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) [15], 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 et al. (2023) [16], who confirmed that the dominant species within the genus *Hyalomma* in goats examined in Basra Governorate was *H. anatolicum*.

During a thorough review of the study carried out by AbdulKarim et al. (2023)[16] of the research in different parts of Iraq, it was discovered that the genus *Hyalomma* is more widespread than others based on ticks

since it was present in most parts of the state with a high level of infection and broad diversity, which speaks in favor of the adaptability of the genus to the conditions of different environments. Their prevalence is due to The suitability of the environmental and climatic conditions to their requirements, and high adaptability, which is further increased by their great diversity in comparison with other species. Table (1) demonstrates the outcomes of number of dead ticks under each concentration or the antiparasitic effect of the aqueous extract of thyme at graded concentration (12.5, 25, 50 and 100 mg/ml) and exposure of ticks to the antiparasitic aqueous extract of thyme of half an hour, 1 hour, 3 hours, 6 hours, 12 hours, and 24 hours. The findings indicated that the aqueous extract was lethal to the ticks just like the traditional acaricide (Cypermethrin) with greater activity with longer exposure and higher concentration. Based on Table (1) it can be seen that the 0% tick-killing effect was recorded after half an hour of exposure to the aqueous extract of thyme at concentrations (100 and 50 mg/ml) relative to the positive control (0%), and the negative control (0%). The aqueous extract however was 0% effective in killing the tick after 24 hours of exposure. The concentration of 100 mg/ml was found to have a significant impact in the killing of the ticks by (66.67) when compared to the positive control (16.67) and the negative control (0).

In the present research, the researchers found out that thyme aqueous extract was lethal to ticks, similar or even more so than the insecticide deltamethrin. The findings revealed that mortality levels were 66.67%, 50%, 33.33 and 16.67 percent, 24 hours after exposure to the extract at different concentrations of 100, 50, 25 and 12.5mg/ml respectively, compared to 16.67 percent in the positive control group. On the one hand, the results also showed that the longer the time spent in contact with the tick, the higher the concentration of the aqueous extract of thyme, and vice versa, on the other, the higher the rate of mortality. These findings are in accordance with other research, which has proved that certain plants possess medicinal properties, such as anti-tick or indicative of a number of prior studies [17]. Thyme is considered one of the most researched medicinal plants in the world, and its application in the treatment of various diseases in people and animals was mentioned [18]. As seen in the vision of the researcher, this study could possibly be the first study in Iraq where the use of thyme aqueous extract as an anti-Tick is investigated since no prior research has been published in this area, in the local context.

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
	_ control	%0	30	0	0
	+ control	0.001	30	0	0
	aqueous extract of thyme	100	30	0	0
		50	30	0	0
1 hour		25	30	0	0
		12.5	30	0	0
		0	30	0	0
		0.001	30	0	0
3 hours	aqueous extract of thyme	100	30	0	0
		50	30	3	10

6 hours		25	30	0	0
		12.5	30	4	13.33
		_ control	30	0	0
		+ control	30	0	0
	aqueous extract of thyme	100	30	0	0
		50	30	3	10
		25	30	2	%6.67
		12.5	30	2	%6.67
		0	30	0	0
		+ control	30	0	0
	aqueous extract of thyme	100	30	0	0
		50	30	3	0
		25	30	2	%6.67
		12.5	30	2	%6.67
		0	30	0	0
		+ control	30	0	0
12 hours		100	30	20	%66.67
		50	30	5	%16.67
		25	30	15	50
		12.5	30	10	33.33
	aqueous extract of thyme	0	30	0	0
		+ control	30	5	%16.67
		100	30	20	%66.67
		50	30	5	%16.67
		25	30	15	50
		12.5	30	10	33.33
		0	30	0	0
		+ control	30	5	%16.67
	aqueous extract of thyme	100	30	20	%66.67
		50	30	5	%16.67
		25	30	15	50
		12.5	30	10	33.33
24 hours		0	30	0	0
		+ control	30	5	%16.67
	aqueous extract of thyme	100	30	20	%66.67
		50	30	5	%16.67
		25	30	15	50
		12.5	30	10	33.33
		0	30	0	0
		+ control	30	5	%16.67
	aqueous extract of thyme	100	30	20	%66.67
		50	30	5	%16.67
		25	30	15	50
		12.5	30	10	33.33
		0	30	0	0
		+ control	30	5	%16.67
	aqueous extract of thyme	100	30	20	%66.67
		50	30	5	%16.67
		25	30	15	50
		12.5	30	10	33.33

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 deltamethrin, with activity increasing with increasing exposure time and concentration.

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	2 ml	30	0	%0
		3 ml	30	0	%0
		4ml	30	0	%0
		5 ml	30	0	%0
	_ control	0	30	0	%0
	+ control	0.001	30	0	%0
	aqueous extract of thyme	2 ml	30	0	%0
		3 ml	30	0	%0
1 hour		4ml	30	0	%0
		5 ml	30	0	%0
	_ control	0	30	0	%0
	+ control	0.001	30	0	%0
3 hours	aqueous extract of thyme	2 ml	30	0	%0
		3 ml	30	0	%0
		4ml	30	0	%0
		5 ml	30	1	%3.33
	_ control	0	30	0	%0
	+ control	0.001	30	0	%0
6 hours	aqueous extract of thyme	2 ml	30	0	%0
		3 ml	30	0	%0
		4ml	30	0	%0
		5 ml	30	1	%3.33
	_ control	0	30	0	%0
	+ control	0.001	30	0	%0
12 hours	aqueous extract of thyme	2 ml	30	0	%0
		3 ml	30	0	%0
		4ml	30	0	%0
		5 ml	30	1	%3.33
	_ control	0	30	0	%0

	+ control	0.001	30	0	%0
24 hours	aqueous extract of thyme	2 ml	30	5	%16.67
		3 ml	30	1	%3.33
		4ml	30	1	%3.33
		5 ml	30	2	%6.67
	_ control	0	30	0	%0
	+ control	0.001	30	5	%16.67

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.

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	2 ml	30	0	%0
		3 ml	30	0	%0
		4ml	30	0	%0
		5 ml	30	0	%0
	_ control	0	30	0	%0
	+ control	0.001	30	0	%0
	aqueous extract of thyme	2 ml	30	0	%0
		3 ml	30	0	%0
1 hour		4ml	30	0	%0
		5 ml	30	0	%0
	_ control	0	30	0	%0
	+ control	0.001	30	0	%0
3 hours	aqueous extract of	2 ml	30	0	%0
		3 ml	30	0	%0

6 hours	thyme	4ml	30	0	%0
		5 ml	30	0	%0
	_ control	0	30	0	%0
	+ control	0.001	30	0	%0
	aqueous extract of thyme	2 ml	30	5	%16.67
		3 ml	30	3	%10
		4ml	30	5	%16.67
		5 ml	30	5	%16.67
	_ control	0	30	0	%0
	+ control	0.001	30	0	%0
12 hours	aqueous extract of thyme	2 ml	30	6	%20
		3 ml	30	10	%33.33
		4ml	30	10	%33.33
		5 ml	30	10	%33.33
	_ control	0	30	0	%0
	+ control	0.001	30	0	%0
24 hours	aqueous extract of thyme	2 ml	30	10	%33.33
		3 ml	30	5	%16.67
		4ml	30	15	%50
		5 ml	30	10	%33.33
	_ control	0	30	0	%0
	+ control	0.001	30	5	%16.67

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.

CONCLUSIONS

The major finding of this study is that both gold nanoparticles (AuNPs) and thyme (*Thymus vulgaris*) extract could serve as an acceptable and useful alternative to conventional chemical pesticides as effective ways of controlling and managing ticks. The investigation was able to show that the two substances when individually used were lethal to the tick samples. This therapeutic activity has been found to be dose-dependent and time-dependent, the lethal effect of the gold nanoparticles and the thyme extract was found to increase with the increasing concentration and the time of exposure. Moreover, the study found considerable synergistic effect of gold nanoparticles and thyme extract in combination. A combination of these produced a more powerful overall effect and became more effective than the isolated use of either substance. In particular, the combination of the two extracts enhanced the rate of action and total kill rate of the ticks. This observation proposes a high

likelihood of using this integrated formulation in future tick management approaches which provides a more sustainable approach that is environment friendly.

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