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ORIGINAL ARTICLE

Assessment of Mannose Modified Nanoliposome of Excretory-Secretory Antigens Against Acute Toxoplasmosis in Mice

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ABSTRACT

Background: *Toxoplasma gondii* is an obligate intracellular protozoan that cause the infectious disease toxoplasmosis. Currently, no effective vaccine exists against this parasite for humans. Therefore, this study aimed to evaluate the efficacy of different vaccine candidates including, excreted/ secreted antigens (ESAs), alum-adjuvanted ESAs, mannose-modified nanoliposomes of ESAs, and mannose-modified nanoliposomes of ESAs with alum against *acute T. gondii* (RH strain) infection in BALB/c mice.

Methods: This study started with the preparation of ESAs, and mannose-modified nanoliposomes of ESAs and mice were then immunized separately. Their anti-*Toxoplasma* antibody and iNOS levels in serum were then measured using ELISA before challenging infection. Subsequently, mice were challenged with *T. gondii* tachyzoites. Afterward, we assessed the peritoneal fluid's burden of the parasite, survival time, serological anti-*Toxoplasma* IgG antibodies and iNOS levels, and histopathology of liver and spleen.

Results: The vaccinated groups with either alum adjuvanted ESAs or mannose-modified nanoliposome adjuvanted ESAs showed significant reduction in tachyzoites counts and prolonged survival time in a dose-dependent manner. Additionally, there was a significant increase in both anti-*Toxoplasma* IgG and iNOS levels ($P<0.001$). Furthermore, there was a considerable reduction in the hepatic and splenic pathological alterations, along with fewer numbers of the parasite than mice received ESAs alone. Notably, vaccination with mannose-modified nanoliposome ESAs yielded more promising results compared to alum-adjuvanted ESAs.

Conclusions: mannose-modified nanoliposome may be regarded as an appropriate adjuvant and antigen vehicle system for vaccination against *Toxoplasma.*

Key words: RH *Toxoplasma*; Excreted/secreted antigens; Nanoliposomes; iNOS, Anti-*Toxoplasma* IgG.

INTRODUCTION

Toxoplasmosis, caused by the parasite *Toxoplasma gondii* (*T. gondii*), affects 30–50% of the world's population, posing significant health and economic concerns. Humans can get infected with *T. gondii* through various routes, including congenital transmission, consuming contaminated food or water, blood transfusions and organ transplants, or contact with infected animals [1]. Pregnant women face particular risks due to the possibility of birth defects, including hydrocephalus, intracranial calcifications, microcephaly, fetal growth retardation, and hepatosplenomegaly [2].

T. gondii infection modulates the host immune response to evade destruction. The defense mechanism against toxoplasmosis involves nitric oxide (NO) production through upregulation of iNOS by IFN-γ and TNF-α. However, *T. gondii* can inhibit NO to sustain replication by inducing transforming growth

factor-β1 (TGF-β1), which leads to iNOS destruction and reduced NO levels [3].

While various drugs exist for treating toxoplasmosis, they often require lengthy treatment periods ranging from weeks to a year and can come with serious side effects like blood cell damage, bone marrow suppression, and allergic reactions [4]. Currently, Toxovax is the only commercial vaccine against *T. gondii*, which exists to prevent abortions in sheep. This vaccine is based on an attenuated strain and is not suitable for humans due to safety concerns about potential reversion to virulence [5]. To overcome these limitations, recent years have seen the exploration and integration of various biotechnological strategies into vaccine development processes. Wide experimental vaccination strategies against *T. gondii* infection in mice have been conducted, including parasite antigens, DNA vaccines, protein subunits, and nanoparticles, with promising results. However, there is still a significant obstacle that needs to be overcome before these in vivo results can be applied [6].

Excreted/secreted antigens (ESAs), comprising various proteases and proteins, are believed to play a significant role in disease severity and parasite dissemination. Targeting these antigens could be a promising approach for vaccine development, potentially inducing antibody production to mitigate the threat. However, complete eradication of *T. gondii* through antibody response alone may not be achievable [7].

Currently, aluminum salt, or alum, is the only adjuvant that the US Food and Drug Administration (FDA) has approved for use in human vaccinations. It has been used safely and effectively for more than 70 years [8]. Despite some vaccine adjuvants being more effective than alum, they have not been licensed for use in humans due to their toxicity [9].

Liposomes are emerging as one of the nanoadjuvants with the greatest efficacy for the delivery of the antigen in vaccines. Liposomes ranging up to 50 µm in diameter are composed of a phospholipid bilayer membrane similar to cell membranes, making them biocompatible and readily accepted by the body, and the FDA has approved some formulations [10, 11]. Liposomes and antigen-presenting cells (APCs) can interact to trigger an immunological reaction of Th1 or Th2. Additionally, they work well as an adjuvant for stimulating the body's defenses against different antigens [10]. Nanoparticles (NPs) have emerged as one of the most widely used and successful vaccine delivery methods, and mannose has been used to change some of these vaccination constructs [12]. Applying mannose to the surface of NPs increases the antigen absorption by APCs [13]. Mannose-modified liposomal vaccines can increase vaccine efficiency by reducing interactions with unnecessary cells and tissues, which can reduce side effects while still generating and promoting the desired immune response [14].

Consequently, the aim of this work was to evaluate the efficacy of mannose-modified nanoliposomes of ESAs as a vaccine against acute toxoplasmosis in mice compared to ESAs and alum-adjuvanted ESAs to find the most efficient vaccine.

METHODS

Ethics approval

This study was approved by the Institutional Animal Care and Use Committee of Zagazig University with approval number **ZU-IACUC/3/F/9/2022**

Parasite

The RH strain of *T. gondii* was obtained from Theodor Bilharz Research Institute and harvested by sterile peritoneal lavage of infected mice [15]. Washing the solution with PBS and centrifuging it at 1400 rpm for 10 minutes at 4 ˚C eliminates any peritoneal cells and debris. The parasite count was then assessed in a hemocytometer using light microscopy (×400) to determine the burden of the tachyzoites [16].

Mice

Seventy seven BALB/c mice, weighing 20-25gm and 3-5weeks in age, were used in this study. The mice were got from the Faculty of Medicine's Animal House at Zagazig University. The Zagazig University's

Institutional Animal Care and Use Committee approved the study to guarantee the moral and responsible use of the animals (No. ZU-IACUC/3/F/9/2022).

Preparation of Toxoplasma ESAs

The experiment involved obtaining tachyzoites from the peritoneal fluid of infected mice in a sterile environment. These tachyzoites were then placed in a sterile conical centrifuge tube containing 25 ml of Roswell Park Memorial Institute (RPMI) 1640 medium with 100 mg/mL streptomycin and 100 IU/mL penicillin. Following 3 hours incubation period at 37°C with soft shaking, the liquid samples in the tubes were spun quickly at 1000 xg for 10 minutes, and their resulting supernatants underwent a process of collection, pooling, and filtration using a 0.22 µm millipore membrane filter. The Bradford method was used for estimating the concentration of protein in the antigenic preparation, which was then kept at -20°C till needed [16, 17].

Preparation of mannose-modifed nanoliposome of ESAs

Mannose-modified nanoliposomes were synthesized using the bath sonication approach, as described in Pezzoli et al. [18]. To prepare the immunogen, a clear suspension of mannose-modified nanoliposomes was first dissolved in chloroform. Subsequently, a supernatant containing ESAs was carefully added to the mannose-modified nanoliposome suspension. The combination was then gently mixed and homogenized using a probe ultrasound to ensure thorough dispersion of the antigens throughout the liposome suspension [19]**.** Subsequently, the chloroform was evaporated by using rotatory vacuum evaporation, and the mixture was hydrated with PBS for 15 minutes. Following PBS washing, the Bradford protein assay technique was used to assess the encapsulation efficiency of ESAs in liposomes. % Entrapment = [Total amount of ESAs added - amount of ESAs recovered in supernatants) / Total amount of ESAs added] × 100. The concentration of the ESAs in the liposomes was adjusted to 2µg /50 µl after purification and calculation of % entrapment [20].

To examine the morphology, size, and surface appearance of the nanoliposomes, we used scanning electron microscopy (SEM) (FEI Company, Hillsboro, OR, USA). Zeta sizer (Malvern particle size analyzer, Malvern, UK) to measure the mean diameter and polydispersity index (PDI) of the nanoliposomes.

Alum adjuvant preparation

It involved mixing 10% aqueous aluminum potassium sulphate and 250 mM sodium hydroxide, followed by stirring for 10 minutes, this forms precipitates of aluminum hydroxide. Then centrifuging at 1000 × g for 10 minutes and the supernatant was then removed. The pellets were washed twice in isotonic saline by centrifugation at 1500 × g for 5 minutes. After re-suspending the pellets in 10 ml of sterile isotonic saline, the suspension was kept at 4 °C until used [21].

Immunization and infection challenge

Mice were randomly divided into eleven groups (n = 7 for each group) as follows: G I: control negative (non-infected); G II: control non-vaccinated infected (control positive); G III: vaccinated with alum at a dose of 0.5 mg, then infected; G IV: vaccinated with ESAs at a dose of 10 µg ,then infected; GV: vaccinated with ESAs at a dose of 10 µg + 0.5mg alum, then infected; G VI: vaccinated with ESAs at a dose of 20 µg then infected; G VII: vaccinated with ESAs at a dose of 20 µg + 0.5 mg alum, then infected; G VIII: vaccinated with mannose modified nanoliposomes of ESAs at a dose of 10 µg, then infected; G IX: vaccinated with mannose modified nanoliposomes of ESAs at a dose of 10 µg + 0.5 mg alum, then infected; G X: vaccinated with mannose modified nanoliposomes of ESAs at a dose of 20 µg, then infected; G XI: vaccinated with mannose modified nanoliposomes of ESAs at a dose of 20 µg + 0.5 mg alum, then infected [22].

The experimental animals were administered vaccines at 2-week interval on days 1 and 14 of the experiment. Two weeks after the final vaccination dose on day 28, mice were challenged with 2 x 10^2 tachyzoites of the RH strain by intra-peritoneal injection. Three mice

from each group were sacrificed five days post-infection (PI) on day 33 of the experiment, while other mice were monitored every day to assess the survival rate for three weeks PI.

Assessment of study

Parasite count determination

Peritoneal fluid was taken from the mice on the sacrifice day and then stained with Giemsa stain. In each group of infected mice, the tachyzoites' mean number was determined based on the counting of extracellular tachyzoites in 10 high-power fields (HPF) in each animal [23].

Serological studies

Serum samples were collected from mice on days 21 (seven days previous to the infection challenge) and 33 of the experiment. Anti-*T. gondii* IgG (Catalog No.LS-F10246) and inducible nitric oxide synthase (iNOS) (Catalog No. LS-F4110) were determined using ELISA kits [24, 25].

Histopathological study

Liver and spleen samples were preserved in 10% formal saline solution. The haematoxylin and eosin (H&E) staining technique was applied to the tissue sections [26].

Statistical analysis

SPSS (statistical software for social science) version 25 (IBM, Armonk, NY, USA) was used to tabulate and analyze the acquired data. Quantitative data are represented as mean and standard deviation (SD). A comparison between three or more groups having quantitative variables was tested using the ANOVA (f) test. A p-value of less than 0.05 was considered statistically significant.

RESULTS

Characterization results

SEM results indicated that the formed NPs (**Fig. S1**) were spherical, and appeared without aggregation, and the mannose-modified NPs of ESAs were larger than those of empty NPs, which was consistent with the NP size analysis results. By Zetasizer, the average diameter of empty mannose-modified nanoliposome was 40–60 nm (PDI = 0.56) and that of the mannose-modified nanoliposome of ESAs was 60–80 nm (PDI = 0.31). The calculated entrapment efficiency was 89.11%.

The parasitological results

In comparison to the infected control, the mean number of tachyzoites **(Fig. 1)** was significantly reduced, with an increasing reduction percentage in all vaccinated groups. Alum group (G III) showed the least effect and had the lowest percentage of decrease, while mannose modified nanoliposomes of ESAs (20 µg) + alum group(G XI) had the best outcomes, with the lowest mean number of tachyzoites and the highest percentage of reduction **(Table 1).**

The survival time

The longest survival time was recorded by (GI) and (G XI) with no statistically significant difference between the two groups followed by groups (G IX), (G X) and (G VIII). While the shortest survival time was recorded in groups, (GII) and (GIII) **(Table 2).**

Results of the serological studies

IgG level

All vaccinated groups induced a statistically significant increase in the mean serum IgG before and after challenging the infection compared to G I, G II, and G III. Notably, the levels were the highest in the sera of the G XI **(Table 3; Fig. S2).**

iNOS level

Vaccination induced a significant increase in the mean serum iNOS concentration before and after challenging the infection compared to G II. Mean serum iNOS levels on day 33 showed higher concentrations. The highest levels were detected in the sera of the G XI **(Table 4; Fig. S3).**

Results of the histopathological study

Hepatic tissue examination

Liver tissues from the healthy control mice revealed normal tissue architecture (**Fig. 2a).** While several pathological changes were observed in the infected control group, G II showed clusters of tachyzoites as a pseudocyst of *T. gondii*, mild swelling of hepatocytes and dilated congested capillaries as well as multifocal lymphoplasmacytic cellular infiltration with few neutrophils, **Fig. 2 (b &c)**.

Also, G III showed multifocal inflammatory cells infiltrate and *T. gondii* pseudocyst with many tachyzoites clusters **(Fig. 2d).** Among G IV, G V, and G VI, these pathological changes were mild with *T. gondii* pseudocyst with some tachyzoites as in **Fig. 2 (e to g).** While groups G VII, G VIII, G IX, and G X showed few pathological features with a reduction of the number of tachyzoites, respectively (**Fig. 2 h–k)**. GXI showed no pathological changes, and the architecture was nearly normal (**Fig. 2l).**

Splenic tissue examination

Sections from GI showed normal compact white pulp and red pulp. However, GII exhibited distorted white pulp with multifocal clusters of *T. gondii* tachyzoites and inflammatory cells. In the vaccinated groups, a reduction in the number of tachyzoites, along with tachyzoites engulfed by macrophages and multinucleated giant cells, was detected at different levels according to each vaccinated group. The most marked improvement in splenic pathology was observed in GXI **(Fig. 3).**

Table 1: Mean tachyzoites count in different studied groups

Groups	Mean $\times 10^4$	±	SD	Range			R%
G II	197.00 [a]	±	3.46	193	-	199	
G III	174.00 [b]	±	3.61	170	-	177	12%
G IV	73.00 [c]	±	4.36	70	-	78	63%
G V	50.67 [e]	±	3.06	48	-	54	74%
G VI	55.33 [d]	±	3.79	51	-	58	72%
GVII	42.00 [f]	±	2.00	40	-	44	79%
G VIII	35.67 [g]	±	3.21	32	-	38	82%
G IX	28.00 [h]	±	2.65	25	-	30	86%
G X	28.33 [h]	±	2.08	26	-	30	86%
G XI	13.33 [i]	±	2.52	11	-	16	93%
F-test	1211.243						
P-value	<0.001**						

a, b, c, d, e, f, g, h & i: Significant difference between any two groups, within the same column have different superscript letter. R%: Reduction percentage**,** **p: Highly significant difference.

G II: control non-vaccinated infected, G III: alum. G IV: ESAs (10 µg); GV: ESAs (10 µg) + alum; GVI: ESAs (20 µg); G VII: ESAs (20 µg) + alum, G VIII: mannose modified nanoliposomes of ESAs (10 µg), G IX: mannose modified nanoliposomes of ESAs (10 µg) + alum, G X: mannose modified nanoliposomes of ESAs (20µg), G XI: mannose modified nanoliposomes of ESAs (20 µg) + alum

Table 2: Survival time post infection (in days)

Groups	Mean	±	SD
G I	17.43 [a]	±	1.90
G II	5.86 [f]	±	0.90
G III	6.86 [f]	±	1.07
G IV	9.71 [e]	±	1.11
G V	12.57 [d]	±	1.90
G VI	12.29 [d]	±	1.80
G VII	15.00 [c]	±	1.41
G VIII	15.14 [b, c]	±	1.35
G IX	15.57 [b, c]	±	1.13
G X	15.14 [b, c]	±	1.77
G XI	16.86 [a, b]	±	2.54
F-test	41.92		
P-value	<0.001**		

[a, b, c, d, e & f]: No significant difference (P>0.05) between any two groups, within the same column have the same superscript letter.

Table 3: IgG levels (OD at wave length 450nm) on days 21and 33 of the experiment

Groups	Day 21			Day 33		
	Mean	**±**	**SD**	**Mean**	**±**	**SD**
GI	0.17 [f]	±	0.02	0.18 [h]	±	0.03
G II	0.82 [f]	±	0.09	1.71 [g]	±	0.28
G III	0.70 [f]	±	0.16	1.98 [g]	±	0.19
G IV	1.48 [e]	±	0.63	5.19 [f]	±	0.74
G V	2.32 [e]	±	0.47	8.21 [d, e]	±	0.71
G VI	3.80 [d]	±	0.61	7.33 [e]	±	1.41
GVII	5.92 [c]	±	0.62	9.31 [d]	±	1.07
GVIII	6.61 [b, c]	±	0.54	11.30 [c]	±	0.98
G IX	7.33 [b]	±	0.55	15.11 [b]	±	0.85
G X	6.64 [b, c]	±	1.10	14.29 [b]	±	1.24
G XI	9.80 [a]	±	1.13	21.59 [a]	±	1.35
F-test	78.66			150.58		
P-value	<0.001**			<0.001**		

[a, b, c, d, e, f, g & h]: No significant difference (P>0.05) between any two groups, within the same column have the same superscript letter. ****:** Highly significant difference

Table 4: iNOS (ng/ml) level on days 21 and 33 of the experiment

Groups	Day 21			Day 33		
	Mean	**±**	**SD**	**Mean**	**±**	**SD**
G I	0.86 ^{e}	±	0.02	1.44 ^{g}	±	0.33
G II	1.31 ^{e}	±	0.21	2.74 ^{g}	±	0.24
G III	1.42 ^{e}	±	0.25	2.90 ^{g}	±	0.30
G IV	2.81 ^{d}	±	0.17	6.77 ^{f}	±	1.12
G V	3.80 ^{d}	±	0.76	11.10 $^{d, e}$	±	1.35
G VI	3.64 ^{d}	±	0.56	9.52 ^{e}	±	1.77
G VII	5.24 ^{c}	±	0.83	13.57 $^{c, d}$	±	1.69
G VIII	7.95 ^{c}	±	0.58	15.40 ^{c}	±	1.97
G IX	9.67 ^{b}	±	0.58	20.57 ^{b}	±	2.18
G X	9.36 ^{b}	±	0.84	19.43 ^{b}	±	2.21
G XI	12.65 ^{a}	±	0.84	26.40 ^{a}	±	1.97
F-test	138.19			81.89		
P-value	<0.001**			<0.001**		

$^{a, b, c, d, e, f \& g}$: No significant difference (P>0.05) between any two groups, within the same column have the same superscript letter.

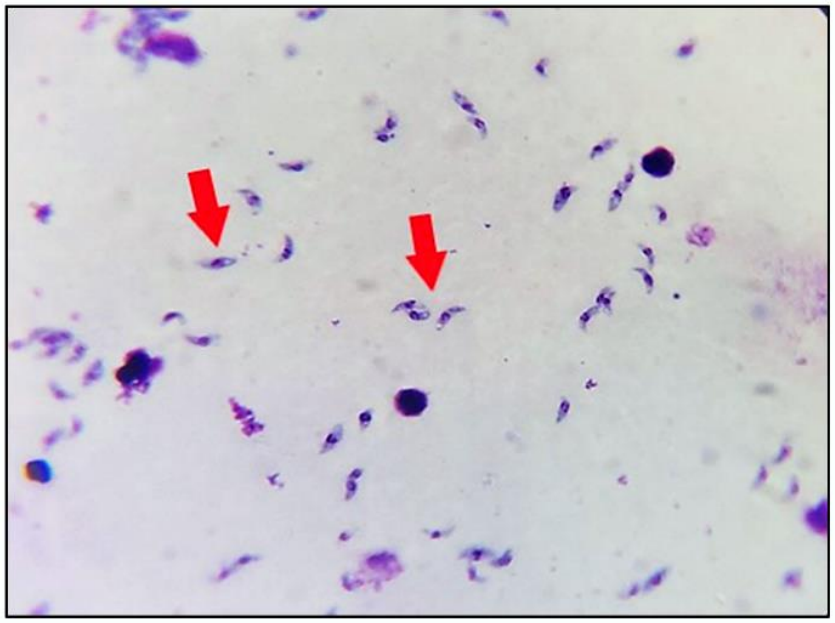

Fig.1: Giemsa-stained peritoneal fluid smear exhibited *T. gondii* tachyzoites clusters (X 1000)

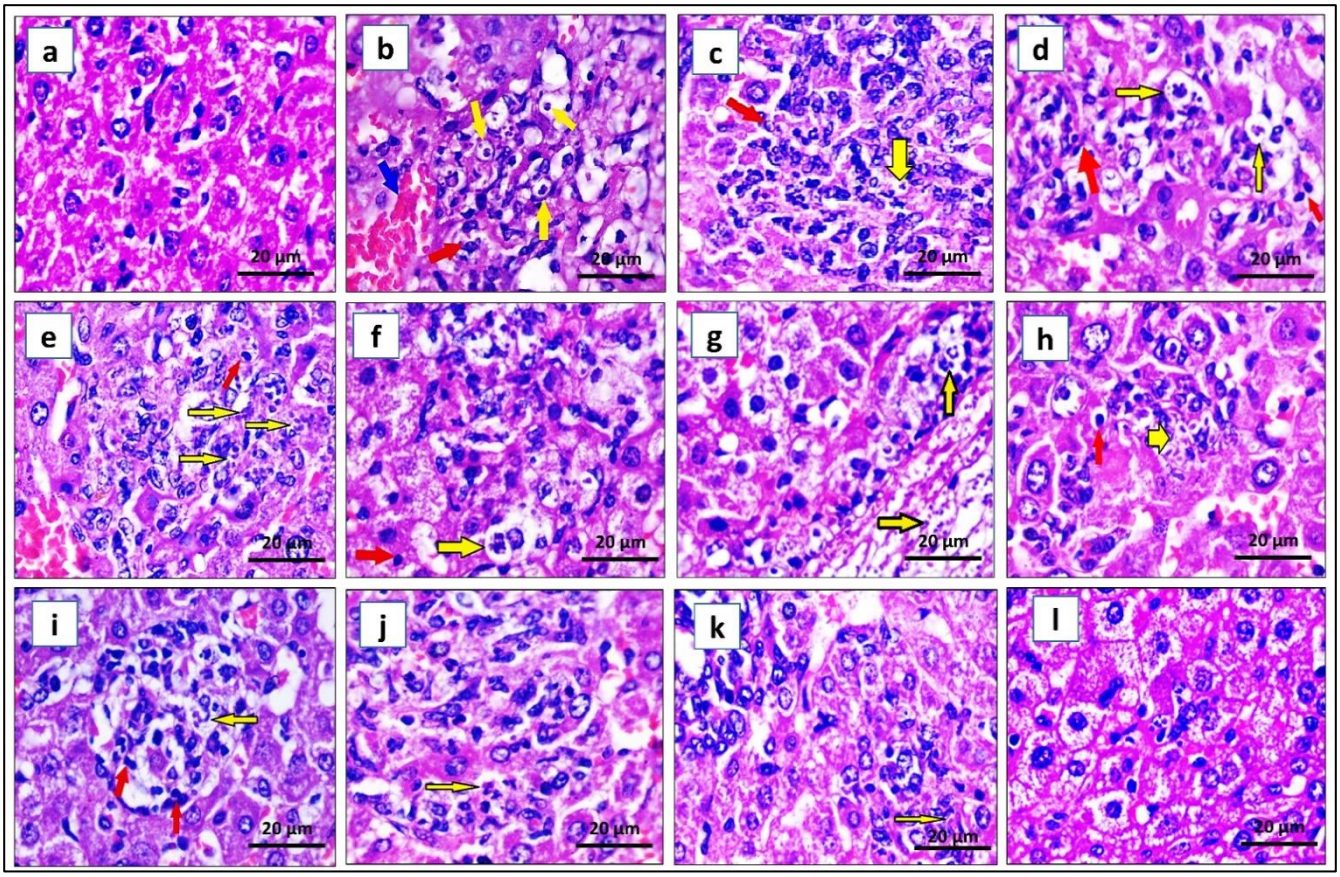

Fig. 2: Sections in the liver of the studied mice groups (H&E, X1000), (a): G I showing normal hepatic architecture with normal sized hepatocytes, **(b & c):** G II showing *T. gondii* pseudocyst with many tachyzoites clusters (yellow arrows), with inflammatory cells infiltrate with necrosis (red arrow) and with dilated congested capillaries (blue arrow), (**d):** G III showing *T. gondii* pseudocyst with many tachyzoites clusters (yellow arrows) with inflammatory cells (red arrows), **(e):** G IV showing *T. gondii* pseudocyst with many tachyzoites clusters (yellow arrows) with inflammatory cells (red arrow), **(f):** G V showing many tachyzoites (yellow arrows) with lymphocytes (red arrow), **(g):** G VI showing tachyzoites (yellow arrows) in and near the liver capsule, **(h):** G VII showing tachyzoites (yellow arrow) with lymphocytes (red arrow), **(i)** G VIII showing tachyzoites (yellow arrow) with few lymphocytes (red arrow), **(j):** G IX showing occasional tachyzoites (yellow arrow) and reduction in inflammatory infiltrates, **(k):** G X showing occasional tachyzoites (yellow arrow) with some lymphocytes and greatest reduction in inflammatory infiltrates, **(l):** G XI showing unremarkable pathological changes with nearly normal liver

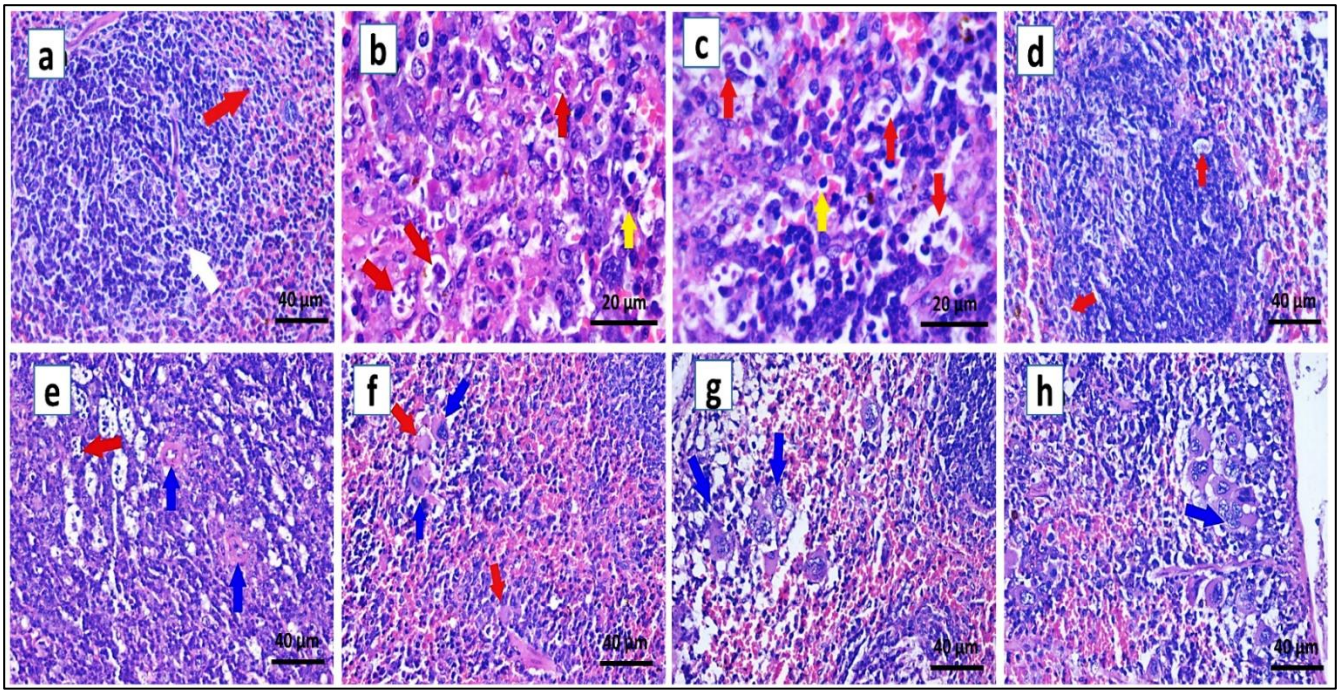

Fig. 3: Sections in spleen of the studied groups. **(a):** GI showing normal splenic architecture with normal compact white pulp (white arrow) and red pulp (red arrow) (H&E, X400), **(b):** GII revealed multifocal clusters of tachyzoites as a pseudocyst of *T. gondii* (red arrows) and plasma cell (yellow arrow) (H&E, X1000), **(c):** GIII showing many clusters of tachyzoites in the white bulb (red arrows) and plasma cell (yellow arrow) (H&E, X1000)., **(d):** GIV showing many tachyzoites (red arrows), **(e):** GV revealed presence of multifocal clusters of tachyzoites (red arrow) and macrophages engulfing many tachyzoites (blue arrows) (H&E, X400)., **(f):** GVI and G VII showed macrophages engulfing tachyzoites (red arrow) and multinucleated cells (blue arrows) (H&E, X400), **(g&h):** Spleen sections of groups vaccinated with mannose nanoliposomes of ESAs showing multinucleated giant cells (blue arrows) and undetectable tachyzoites (H&E, X400)

DISCUSSION

To find a suitable candidate vaccine to counteract *T. gondii* infection, numerous studies have been conducted. ESA is a significant *T. gondii* antigen due to its expression in both the acute and chronic phases of infection [7]. Rhoptries, dense granules, and micronemes all secrete ESAs. ESAs improve cell-mediated immune response, and they are crucial in long-term immunity induction in healthy individuals with chronic infections. As such, it represents a strong candidate for *T. gondii* vaccine. Still, adjuvants are required to increase the efficiency of the vaccine as it stands [27].

Liposomes are well-defined structures, they can be used to improve formulations by attaching targeted ligands, adding additional adjuvants, and changing the membrane composition [10]. According to earlier research, the vaccine's effectiveness increased once it was liposome-encapsulated [28]. In the current work, to increase the effectiveness of the nanoliposomes and stimulate the immune system even more, mannose molecules are added to their surface. It has been suggested in numerous studies targeting the delivery of drugs, vaccines, and gene therapy [29]. Mannosylated liposomes improved the delivery of antigen to certain immunological cells, which increased lymphocyte activation, strengthened the Th1-biased humoral response, and promoted the activity of CD4+ and CD8+ T cells, resulting in a rise in effector cytokine production [30].

Concerning parasitological assessment, our results showed that the mean number of *T.*

gondii tachyzoites decreased in a very statistically significant way in all vaccinated groups compared to the infected control mice. Additionally, mannose modified nanoliposomes of ESAs were more potent than ESAs alone. Moreover, the ESA vaccine's potency was greatly increased when combined with mannose-modified nanoliposomes and an alum mixture. Alum was employed as an adjuvant and was found to be quite beneficial. However, it produces a limited cell-mediated response and frequently results in unfavourable immunoglobulin reactions. Additionally, it might result in fever and significant local tissue irritation [31].

The reduction in the parasite counts is attributed to strong stimulation of humoral and cellular immune responses and the development of anti-ESA antibodies that not only mediate parasite lysis but also agglutinated tachyzoites. Also, the generated protective antibodies help to the earlier activation of the complement system, aiding phagocytosis with the clearance of parasites in the organs and parasitemia [32]. These observations were in agreement with Costa-Silva et al. [33], who reported that the animal's survival rate was elevated after receiving an ESA vaccination by lowering the parasitemia. Also, the immunized mice with nanoliposomal ESAs showed a decreased parasite burden in comparison to the PBS and ESA groups [28].

Concerning survival time, there was a significantly prolonged survival time in all vaccinated groups except the alum vaccinated. This could be attributed to vaccination lowering tachyzoites' vitality, leading to prolonged survival time. The longest survival time was in mice that received mannose modified nanoliposomes of ESAs. This agreed with Abdollahi et al. [34] who suggested that the nanoliposome was a more effective way of controlling mortality following *T. gondii* infection. Ezz Eldin et al. also mentioned higher survival rates in mice vaccinated with the autoclaved *Toxoplasma* vaccine (ATV) or ESA plus BCG adjuvant [35]. Additionally, it has been reported that either mice received ESAs alone, ESAs adjuvanted with alum, or ESAs loaded on alginate-NPs exhibited a statistically significant increase in survival time when compared to the infected control [22].

The results of IgG level showed increased titers in all vaccinated mice. IgG levels were higher in the 5^{th} week than in the 3^{rd} week. This is attributed to adjuvants made of alum, which likely change the kind of acquired immunity to the vaccination antigens into an antibody-mediated immune response (type 2) [36]. This was in agreement with Costa-Silva et al. [33], who reported that ESA can produce a high level of IgG1. In addition, combining the ESA vaccination with the adjuvant can improve humoral immunity [37].

Regarding the levels of iNOS, there was promoted production of iNOS and maintained h igh titres among various vaccinated groups in a statistically significant way. The immune stimulatory potential of nanoliposomes leads to a strong immune response with cytokines production, which is essential for infection control. Murine macrophages and dendritic cells express iNOS that is essential for mice to control and restrict *T. gondii* development and propagation in vivo through the synthesis of enormous amounts of NO. NO can destroy *Toxoplasma* tachyzoites and kill intracellular infections [38].

This result was consistent with the findings of Czarnewski et al. who observed that immunization with recombinant *T. gondii* expresses the highly immunogenic heat shock protein 70 (rTgHSP70), increased the number of iNOS+ cells in the brain, which is strongly associated with decreased parasite burden and better results were in groups immunized with rTgHSP70 alone or alum-adsorbent [39].

In the present study, several pathological changes were observed in the infected control. These findings are consistent with previous studies [22, 40]. Among the vaccinated groups, the pathological features of examined tissues were reduced with a reduction of inflammatory changes and a reduction of the number of tachyzoites. Notably, G XI showed nearly normal tissues. This was consistent with finding

of Ezz Eldin et al. [35] and Gaafar et al. [22], who reported that vaccinated groups with ESA have shown a significant decrease in the hepatic and splenic pathological alterations found. It also observed that the mice that got nanovaccine formulations had the greatest reduction in inflammatory infiltrates and the degree of necrosis [32].

CONCLUSIONS

Using mannose modified nanoliposomes along with alum as adjuvants to ESAs for vaccination against RH *Toxoplasm*a induced the highest significant results regarding reduction of tachyzoites, increasing anti-*Toxoplasma* IGg, and iNOS levels, with no evidenced pathological changes. Mannose modified nanoliposome NPs were found to be a better adjuvant than conventional alum in enhancing the immunogenicity of ESAs against acute toxoplasmosis.

Conflict of interest: None

Financial Disclosures: None

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Supplementary File

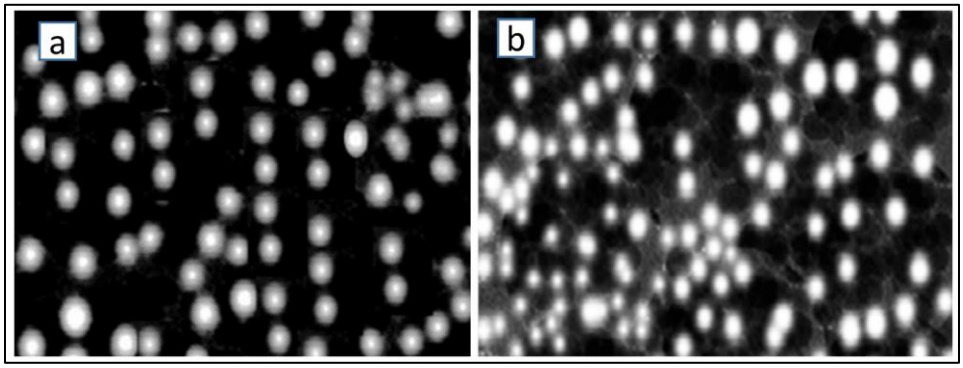

Fig. S1: Images of SEM; a: Nanoliposome, b: Nanoliposome loaded with ESAs

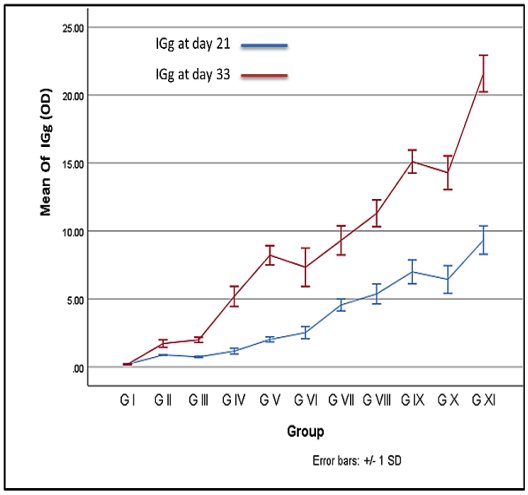

Fig. S2: Comparison between mean IgG levels on days 21and 33 of the experiment in different studied groups

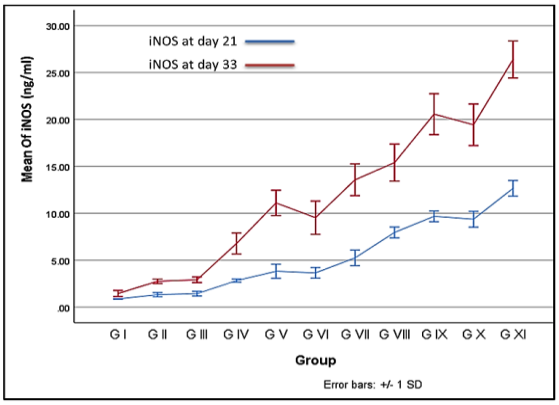

Fig. S3: Comparison between mean iNOS levels on days 21 and 33 of the experiment in different studied groups

Citation

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