Characterization of Membrane-associated Progesterone Receptor Component-2 (*MAPRC2*) From *Trichinella spiralis* and Its Interaction With Progesterone and Mifepristone

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Abstract

Background: *Trichinellosis* is a food-borne zoonotic disease caused by nematode viz., *Trichinella spiralis*. Physiologically, the high progesterone (P4) doses cause new borne larvae (NBL) mortality in *the parasite, while the low doses maintain pregnancy. In contrast,* Mifepristone (RU486) works as an antagonist against the progesterone receptor (PR) and possesses abortifacient activities.

Methods: In the present study, *T. spiralis* membrane-associated progesterone receptor component-2 (*Ts-MAPRC2*) gene was cloned and characterized by protein sequencing. Furthermore, the expression, purification, immunoblot assay, binding ability with progesterone antibody, and immunofluorescence assay were performed. A direct effect of progesterone (P4) and mifepristone (RU486) on the *Ts-MAPRC2* gene was determined using *in-vitro* cell culture that showed different expression levels at all developmental stages [muscle larvae (ML), female adult worm (F-AL), male adult worm (M-AL) and new borne larvae (NBL)]. Subsequently, the *in-vitro* phenotypic effect of P4, RU486, and *rTs-MAPRC2-Ab* on F-AL and ML stages were measured. Later on, the *in-vivo* phenotypic effect and relative mRNA expression of mifepristone on the F-AL stage were studied.

Results: Our results revealed that the *Ts-MAPRC2* gene is critical to maintaining pregnancy in the female adult worm (F-AL) of *T. spiralis*. The P300 ng/mL of P4 and M100 ng/mL of RU486 showed downregulation of the *Ts-MAPRC2* gene in F-AL ($P \leq 0.05$). This plays an important role in abortion and possibly decreases the worm burden of *T. spiralis* in the host. Only P30 ng/mL showed significant upregulation in F-AL ($P \leq 0.05$).

Conclusions: The current study provides new insights regarding the antihormone (P4 & RU486) drug design and vaccine therapy of recombinant *(rTs-MAPRC2)* protein as well as their combined effects to control *T. spiralis* infection.

Background

Trichinellosis is an important food-borne zoonotic disease caused by a nematode parasite named *Trichinella spiralis* (*T. Spiralis*), it ranked 7th among the world's most infectious parasitic diseases (1). The use of pork meat in various forms (i.e., raw, undercooked, etc.) and its by-products are the main source of infection in humans (2–4). Since China is the leading consumer of pork and its by-products (88.1 pounds per capita) where high morbidity from this disease due to the expanded dissemination of naturalized animals and human reserves increasingly becoming a serious issue (5–7). Although anti-helminthic agents are extensively practiced against *Trichinellosis*, their excessive use triggers the emergence of drug residues in meat, drug resistance in the parasite, and it adversely affects the environment as well. Thus, the advancement of an efficient vaccine against *Trichinellosis*, especially for humans and pigs, is a potential measure to prevent infection (7, 8). Recently, a series of proteins participated against host irruption, the viability of parasite, and therefore produced resistance as vaccine applicants. Further, their defensive effect resists *T. Spiralis* larvae to inoculate in model animals have been examined (9–12). Many types of these vaccines showed relatively positive action against *T. Spiralis* infection; yet, no such vaccine commercially

available that provides adequate immunity against *T. Spiralis* infection (1). In recent years, the use of hormonal-based drugs is being considered a novel and effective alternative that offers appealing approaches against parasitic diseases. Progesterone (P4) is a gonadal hormone synthesized in the female ovary, male adrenal cortex, testes, and its levels are high in females, especially in the follicular phase of the menstrual cycle as compared to males (13). P4 also has immunosuppressive effects and shifts in immune response from type 1 T helper (Th1) to type 2 T helper (Th2) cells (14, 15). Anzaldúa et al. (16) studied the highest level of P4 that causes resistance against parasite invasion during pregnancy. Physiologically, the association between the increase in the P4 doses and induction of NBL mortality was observed in *T. spiralis* and *T. zimbabwensis* (17, 18).

On the other hand, mifepristone (RU486) works as an antagonist against the progesterone receptor (PR) and glucocorticoid receptor (GR) with abortifacient and anticancer activities (19). P4 acts by non-genomic pathways mediated with membrane-binding progesterone, including membrane progesterone receptors (mPRs), progesterone receptor membrane component-1(*PGRMC-1*), progesterone receptor membrane component-2 (*PGRMC-2*), and specified in humans (20, 21). *PGRMC-2* is expressed in many tissues, especially expressed in the placenta with other reproductive tissues, and also non-reproductive tissues (liver and nervous tissues). Surprisingly, this receptor membrane*(PGRMC-2)* was also found in another organism like *Caenorhabditis elegans* (*C. elegans*), illustrated to express an analogous protein (vem-1) in mammals (22). In the case of helminths, a few studies present research related to *PGRMC* receptors (23). Prior studies observed different proteins related to sex hormone receptors comprising *PGRMC*, progestin-induced protein, p48 progesterone-receptor-associated protein, small androgen receptor-interacting proteins that were found in *Schistosoma japonicum* (24, 25). Likewise, mifepristone (RU486), was one of the first medications approved for surgical abortion, it is often used to terminate an early or mid-term pregnancy (26). Hence, progesterone receptor (PR) and binding type of P4 molecules (agonist) and RU486 (antagonist) might be helpful to elaborate *T. spiralis* species regarding differentiation and reproductive development as well as creating potential pharmacological targets that might be used as a drug therapy against *Trichinellosis*.

The purpose of this study was to clone and characterize the *T. spiralis* membrane-associated progesterone receptor component-2 (*Ts*-MAPRC2) gene. Subsequently, we studied the protein sequencing, expression, purification, immunoblot assay, immunofluorescence assay (IFA), and binding ability with Progesterone (P4). Further, this study used an *in-vitro* cell culture technique to find out the direct effect of progesterone (P4) and mifepristone (RU486) on *Ts-MAPRC2*, which showed different expression levels of *Ts*-MAPRC2 gene at all developmental stages [female adult worm (F-AL), male adult worm (M-AL), muscle larvae (ML), new borne larvae (NBL)] of *T. spiralis*. Similarly, the *in-vitro* phenotypic effect of P4, RU486, and r*Ts*-MAPRC2-Ab (rat- anti-sera- against *rTs-MAPRC2*) on F-AL and ML stages were observed. Additionally, the *in-vivo* phenotypic effect and relative mRNA expression of mifepristone (RU486) on the F-AL stage were studied. This approach will help to design new antihormone (P4 & RU486) drug and vaccine therapy of recombinant (r*Ts*-MAPRC2) protein with their combined effect to control *Trichinellosis*.

Methods

Animals and Parasites Preservation

BALB/c mice (body-weight 18-20g) and SD (Sprague-Dawley) rats (body-weight 220-250g) were bought from *Qinglongshan*, Animal breeding farm, Nanjing, Jiangsu P.R. China (certified: SCXK 2008-0004); maintained under supervised condition by Animal House of Nanjing Agricultural University. *T. spiralis* (ISS534) utilized in this experiment was isolated from a pig in Nanyang, Henan Province, China, and were preserved by serial passage in BALB/c mice after every 6–8 months. *T. spiralis* muscle larvae (ML) were restored from BALB/c mice by 40 dpi (days post-infection) with standardized HCl-pepsin digestion technique (27). Adult worms (AL) were retrieved from intestine at 6 dpi, and new borne larvae (NBL) were recovered from a female adult at 6 dpi from the RPMI-1640 culture media at 37°C for 24 h as previously described by (28, 29). The parasite collected at different development phases were homogenized and chilled in liquid nitrogen.

Sequence Analysis of Ts-MAPRC2

The whole-genome coding sequence of the *Trichinella spiralis (Ts)* membrane-associated progesterone receptor component-2 (Ts-MAPRC2) gene (GeneBank Accession No. XM_003375886.1) was primarily obtained from the online GeneBank, The National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/). Sequence properties of *Ts*-MAPRC2 were studied from the Expasy website (http:// www.expasy.org/). The Phylogenetic analysis of the MAPRC2 (*Trichinella spiralis*) protein sequence was carried out with identical genes from the other strains using the Clustal Omega (https://www.ebi.ac.uk/Tools/msa/clustalo/). Prediction of transmembrane in protein and N-terminal Signal peptide prediction were confirmed by online tools TMHMM (http://www.cbs.dtu.dk/services/TMHMM/) and SignalP-5.0 Server (http://www.cbs.dtu.dk/services/SignalP/).

Cloning of Ts-MAPRC2

The full-length sequence of *Ts-MAPRC2* comprises 234 amino acids (aa) (705 bp). Fragment size of 97-234aa (225-705bp) from the *Ts-MAPRC2* having conserved domain (104-173aa) was expressed in the current study. The *Ts-MAPRC2* gene of *T. spiralis* was amplified using RT-PCR analysis. Specific sense (5'-GAATTC AATAGATTTCGTATAAAATGGACATCT-3') and anti-sense (5'- AAGCTT TCACTGATCATCAACATCACAATCAGAG – 3) primers were used with restriction enzymes *EcoR Ⅰ* and *Hind III*. The amplified PCR materials were electrophoresed and cleansed through Gel-Extraction Kit (Omega, USA) and eventually ligated into cloning vector pMD19-T (TaKaRa, China). The recombinant (pMD19-T/ *Ts-MAPRC2*) plasmid was further processed into *E. coli* (DH5α) strain (Invitrogen, China) and cultivated in LB (Luria Bertani) medium with ampicillin (100 µg/mL). Positive bacterial (pMD19-T/ *Ts*-MAPRC2) clones were assured by digestion of restriction enzymes and confirmed through sequencing (Invitrogen, China).

Development of recombinant Ts-MAPRC2 (rTs-MAPRC2)

The restriction digestion of the plasmid (pMD19-T/ *Ts-MAPRC2*) was carried out by using the enzymes viz., *EcoR Ⅰ*, and *Hind* III were cloned into the prokaryotic expression vector pET-32a (+) (Novagen, USA).

Recombinant plasmid (pET32a (+)/ *Ts-MAPRC2*) was then processed into BL21 (DE3) and induced protein expression by 1 mM IPTG (Isopropyl-β-D-thiogalactopyranoside) (Sigma-Aldrich). Further, cells were harvested and lysed by lysozyme (10 µg/mL) (Sigma-Aldrich, USA), pursued by sonication. The sonicated outputs were subsequently confirmed on the SDS-PAGE (12% w/v). Recombinant *Ts-MAPRC2* (*rTs-MAPRC2*) protein was purified by the His-TrapTM FF column following the manufacturer's instructions (GE Healthcare, USA), and protein concentration was calculated by Pierce-TM BCA-Protein Assay Kit (Thermo Scientific, USA). Also, empty pET32a (histidine-tagged) protein was purified and expressed using the same procedure mentioned above for *Ts-MAPRC2*, and utilized vector-protein as a negative control. Pictures of the SDS-PAGE carrying the r*Ts-MAPRC2* purified protein were taken. The stock of r*Ts*-MAPRC2 protein was prepared and preserved at − 80°C until the next experiments.

Generation of the rat-polyclonal antibody of rTs-MAPRC2

For anti-sera preparation, SD-rats (n = 3) were immunized subcutaneously with *rTs-MAPRC2* protein (300 µg) combined with Freund's complete adjuvant (Sigma–Aldrich, Darmstadt, Germany) equally. After two weeks, the second dose of 200 µg *rTs-MAPRC2* protein was inoculated with Freund's Incomplete Adjuvant (Sigma–Aldrich). Again, we repeated the same two booster doses at an interval of one week. After one week of the last dose, serum samples were collected and stored at − 80°C for further experiments. Serum collected from non-treated rats (n = 3) was used as a negative control.

Immuno-blot assay of rTs-MAPRC2

Separation of *rTs-MAPRC2* protein carried by SDS-PAGE (12%), and were transferred to nitrocellulose membrane (Millipore, USA). Afterward, the membrane was blocked with skim milk (5% w/v) powder in TBST (0.5% Tween-20 in TBS) at 37°Cfor 2h. Then washed three times with TBST, and membrane again incubated with primary antibody (rat- anti-sera- against *rTs-MAPRC2*) at 37°C for 1h (1:300 dilution). For negative control, we used the normal rat serum. Following this, the strips were washed three times and again incubated with secondary antibody conjugated-Horseradish peroxidase (HRP) goat-anti-rat IgG (1:3000 dilutions) (Sigma, St. Louis, MO, USA) for 2h at 37°C. In the end, the immune-reaction were appeared within 3–5 min, as per the manufacturer's directions of the DAB-HRP color development kit (Beyotime, China).

Determine binding ability of rTs-MAPRC2 **protein with progesterone**

In order to determine the binding ability of *rTs-MAPRC2* protein with progesterone, we used the progesterone antibody (PROG-Ab) kit (Sandwich ELISA kit, FY95030-B, Feiya-Biotechnology, China), the pET-32a and PBS (phosphate buffer saline) were used as a control for the relative comparison. Initially, a standard curve was prepared using different concentrations (ng/mL) of the standard sample. According to kit instructions, 50 µl of the standard sample was added into three wells. Sample diluent (40 µl) was combined with *rTs-MAPRC2* protein (10 µl) and pET-32a (10 µl) protein and incubated at 37°C for 30 min. After this, it was washed with the diluted washing solution (20x) 5 times for 30 sec each. Then 50 µl HRP-Conjugate solution was poured in each well then incubated again at 37°C for 30 min. Again, it was washed

with the diluted washing solution (20x) 5 times for 30 sec. Afterward, the 50 µl Chromogen-A and Chromogen-B were added and incubated for 10 min at 37°C. Finally, 50 µl of a stop solution was added, and the color change from blue to yellow was observed. The optimum density (OD) values were measured at 450 nm with a plate reader and compared (Thermo Fisher, Life Technologies).

Immunofluorescent assay of rTS-MAPRC2 at the different developmental stage

The cross-section of *T. spiralis* samples at different developmental stages, i.e., ML, F-AL, M-AL, & NBL were fixed in 4% formaldehyde-0.2% glutaraldehyde with PBS for 45 min, and liquid nitrogen was used as a frozen snap. With the use of a cryotome (CM1950-Frankfurt, Germany), worms were cut into cross-section pieces (10 µm thick) and cleansed by PBS. First, the 5% BSA (Bovine serum albumin) was treated with slides to block non-specific binding and continued by incubation with primary antibody (rat- anti-sera-against *rTs-MAPRC2*) as well as normal rat serum (control group) at 37°C for 2 h (1:300 dilution). Then both group slides were washed with PBS then incubated with Cy3-goat labeled anti-rat as a secondary antibody (Beyotime-Shanghai, China) for 1h at 37°C. Thereafter, the DAPI (diamidino-2-phenylindole) (Sigma, USA) stain was used for staining the nuclei of the worm cells for 5 min under darkness. Lastly, a laser confocal microscope (PerkinElmer, USA) was used to observed worm cross-section cells.

Relative mRNA expression of Ts-MAPRC2 gene at different stages incubated with progesterone (P4) and mifepristone (RU486) by in vitro

The powder form of progesterone (P4) and mifepristone (RU486) were purchased from Sigma (Sigma-Aldrich, USA), and dissolved in absolute ethanol to prepare the desired stock solution following company instruction. Furthermore, the stock solution was sterilized by using the filtration process with a 0.2 mm Millipore filter. The high levels of progesterone range were 100–200 ng/mL in rats, 81.9 ng/mL in mice, 25–30 ng/mL in pigs, and 25 ng/mL in pregnant women were determined (30–33). So, five different concentration of agonist progesterone (P300 ng/mL, P100 ng/mL, P30 ng/mL, P10 ng/mL, and P3 ng/mL) and also three concentrations of antagonist mifepristone (M300 ng/mL, M100 ng/mL, and M30 ng/mL) were prepared. Also, control (only RPMI), and control vehicle ethanol (EtOH-RPMI)(0.125%) were prepared described by (34). All the stages of the parasites viz., F-AL, M-AL, ML, and NBL were collected as described previously by (28, 29). The 2000 worms/well at all above parasite stages (ML, AL, NBL) were cultured in 6-well plates, and a 6-well precondition was used. The cultured medium consists of RPMI-1640, 10% heat-inactivated FBS (Fetal bovine serum), and 2% antibiotics (100 U/mL penicillin; 100 mg/mL streptomycin) (Gibco, Paisley, UK), and incubated at the 37°C and 5% of CO_2 for 48 h, and the medium was changed after 24 h for all treatments. The female (F-AL) and male (M-AL) adult worms were separated under light microscopy using Axiovert Zeiss Microscope (25x Neo Plan objectives). Gene (*Ts-MAPRC2*) expression of all treated groups at various developmental stages was measured by relative quantitative PCR (qRT-PCR) as previously described by (35). Briefly, the Trizol technique was used for RNA extraction from treated groups at all stages of worms using a prime script RT reagent kit (Takara, CA, USA). The isolated RNA from each group at all parasite stages were reverse transcribed by using HiScript II Q RT SuperMix (Vazyme, Nanjing, China) kit using manufacturer instructions. The *Ts*-MAPRC2 gene-specific primers were used as follows: forward (5'-ACGATGTGACCCGAAAGAGA-3') and reverse (5'-CATGCATAGCCCATTCACGT-3').

Quantitative amplifications were performed by BI 7500 Fast Real-time PCR System (Applied Biosystem, USA) with the use of Cham-QTM SYBR qRT-PCR master mix-Kit (Vazyme, Nanjing, China). GenBank Accession No. AF452239 GADPH (Glyceraldehyde-3-phosphate dehydrogenase) of *Trichinella* was used as an internal control. The primer designed for GADPH were followed as forward (5'-GTCGTGGCTGTGAATGATC-3') and reverse (5'-GCTGCCCCACTTAATTGCTT-3'), and data were computed using the comparative Ct ($2 - ^{\Delta\Delta Ct}$) technique (35).

In-Vitro **phenotypic effect of P4, RU486, and** rTs-MAPRC2-Ab **on F-AL and ML stages**

In this experiment, the selected concentration of progesterone (P30 ng/mL), mifepristone (M100 ng/mL), and *rTs-MAPRC2-Ab* (rat- anti-sera- against r*Ts-MAPRC2*) ratio (1:300 dilution) were used at F-AL and ML stages with both controls (only RPMI and EtOH-RPMI) collected as described above, and the previously described procedures were followed according to (28, 29). The 2000 worms/well of both stages (F-AL, ML) were cultured in a 6-well plate, and a 6-well precondition was used. The cultured medium consists of RPMI-1640, 10% heat-inactivated FBS (Fetal bovine serum), and 2% antibiotics (100 U/mL penicillin; 100 mg/mL streptomycin) (Gibco, Paisley, UK), and incubated at 37°C and 5% CO_2 for 48 h with medium changed after 24 h for all treatments. The female (F-AL) and male (M-AL) adult worms were separated under light microscopy using Axiovert Zeiss Microscope (25x Neo Plan objectives) and observed the phenotypic appearance at both stages (F-AL and ML) by using an inverted bright field microscope (Olympus, Shibuya, Japan). In the F-AL stage, the pregnancy maintenance or aborted to new borne larvae (NBL) were observed. While at the ML stage ecdysis (molting process) and motility of parasites were noted.

The phenotypic effect and relative mRNA expression of mifepristone on F-AL stage by In-Vivo

To determine the phenotypic effect of mifepristone on F-AL (female adult worm) of *Trichinella spiralis*, a total of 12 BALB/c mice were divided into three groups (i) mifepristone (M100 ng/mL) administration, (ii) adjuvant group EtOH (Olive oil-ethanol 3:1), and (iii) control group. Mifepristone (M100 ng/mL) powder from Sigma (Sigma-Aldrich, USA) was dissolved in a mixture of Olive oil-ethanol (EtOH) and injected subcutaneously (SC) into BALB/c mice every 24 h till retrieved AL (Adult worms) from the intestine at 6 dpi (days post-infection) with 0.1 mL of the suspension comprising boost dose of steroid according to the protocol of González et al. (1997). In the adjuvant group EtOH (Olive oil-ethanol), BALB/c mice were administrated only adjuvant EtOH with the same protocol, and the control group was kept without any drug therapy. On day six of mifepristone (M100 ng/mL) treatment, all groups of mice have orally injected 500 *T.spiralis* muscle larvae (ML) with the help of gastric cannula (27). Adult worms (AL) were collected from the intestine at 6 dpi (days post-infection) and adult worms (F-AL, M-AL) were separated using Axiovert Zeiss Microscope (25x Neo Plan objectives) and observed the phenotypic appearance, especially pregnancy, occurred or not using an inverted bright field microscope (Olympus, Shibuya, Japan). The gene (*Ts*-MAPRC2) expression of all the three groups at the F-AL (female adult worm) developmental stage was also measured by relative quantitative PCR (qPCR) as described above and previously by (35).

Statistical analysis

The data were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's and LSD (Least significant difference) using analytical statistics software (Statistix 8.1, USA, 2003). The qRT-PCR data were normalized using $2^{-\Delta\Delta Ct}$ in Microsoft Excel 2010 (Redmond, Washington, USA). Origin software (Origin Pro 2021) was used to perform figures (Origin Lab Corporation, Northampton, MA, USA). Statistical data were presented as mean ± SD (n = 3). $P \leq 0.05$, $P \leq 0.01$ were considered significant.

Results

Sequence Analysis of Ts-MAPRC2

The full-length gene sequence (Gene Bank Accession No. XM_003375886.1) of 705bp was predicted to encode protein consisting of 1-234aa, as well as possess the conserved domain of104-173aa (Fig. S1). Fragment size of 480bp (between 225–705 bp) of the *Ts*-MAPRC2 gene were used for cloning that encodes the protein of 137aa (97-234aa) possessing the conserved domain of 6-75aa submitted online in NCBI (http://www.ncbi.nlm.nih.gov/) and issued a specific accession number (MT093680). Bioinformatics online tools TMHMM revealed that there were no transmembrane (Fig. S2A) and Signal peptide (Fig. S2B) found in *Ts-MAPRC2* (MT093680). Percent matrix index (Fig. S2C) showed more similarity of the *Ts-MAPRC2* with other trichinella species strains. Phylogenetic tree and multiple sequence alignment of *Ts-MAPRC2* by using the Clustal Omega with other trichinella species are presented in Fig. 1.

Cloning of Ts-MAPRC2

The amplified PCR product of the *Ts-MAPRC2* gene (480bp) was procured successfully from cDNA of *T. spiralis* (Fig. 2A) by using the pair of primers, and cloned into pMD-19T (cloned vector). Then transformed into pET-32a (expression vector) (Fig. 2B), and confirmed by restriction enzymes digestion using the *EcoR Ⅰ* and *Hind III.*

Expression, purification, and Immunoblot of rTs-MAPRC2

Cloned *Ts-MAPRC2* gene (480bp) sequences were translated into 137aa, and molecular weight was estimated to 17.6 ≈ 18 kDa. The *rTs-MAPRC2* protein was expressed on SDS-PAGE (12%) with IPTG and observed high expression after five induction hours (Fig. 3A). After purification, the molecular weight was showed around 38kDa (Fig. 3B, Lane 1) along with 20 kDa mass of (poly his-tag pET-32a) expression vector (Fig. 3B, Lane 2). Immuno-blot assay revealed that *rTs-MAPRC2* could be recognized by polyclonal antibodies produced in rat serum against the *rTs-MAPRC2* protein (Fig. 3C, Lane 1), and compared with normal rat serum (Fig. 3C, Lane 2). The above result showed that *rTs-MAPRC2* possess antigenicity, and easily recognized by the host immune system.

Binding ability of rTs-MAPRC2 protein by Sandwich ELISA

The binding capacity of *rTs-MAPRC2* protein with progesterone antibody (PROG-Ab) was compared with pET-32a and PBS as control. Our results revealed that there are highly significant differences ($P < 0.01$)

between *rTs-MAPRC2* protein and both controls (pET-32a and PBS) (Fig. 4) compared to the standard. But no significant difference was observed between pET-32a and PBS.

Immuno-fluorescent assay (IFA) of Ts-MAPRC2 gene at various developmental stages

IFA was performed to determine the presence of the *Ts-MAPRC2* gene at different developmental stages using anti- *rTs-MAPRC2* rat serum, Cy3-conjugated secondary antibody, and DAPI (nuclei dye). The cross-section cells of all developmental stages [ML, F-AL, M-AL, and NBL] of *T. spiralis* were studied. As shown in Fig. 5, Cy3-labeled *T*s-MAPRC2 gene, DAPI-labeled nuclei displayed red and blue fluorescence for all stages cross-section cells (ML, F-AL, M-AL, and NBL) respectively. ML and F-AL stages showed high *Ts*-MAPRC2 gene localization as compared to M-AL and NBL stages. Moreover, F-AL showed high immunolocalization compared to M-AL. However, no red fluorescence was observed in all stages (ML, F-AL, M-AL, and NBL) of the control groups.

Relative mRNA expression of Ts-MAPRC2 at all stages by in-vitro treatment of P4 and RU486

The transcriptional pattern of *Ts-MAPRC2* gene in the parasite treated at different concentration of P4 (P300 ng/mL, P100 ng/mL, P30 ng/mL, P10 ng/mL, and P3 ng/mL) as well as mifepristone (M300 ng/mL, M100 ng/mL, and M30 ng/mL) as well as their multi comparison analyses was investigated to find out the up-and down-regulation at different stages (F-AL, M-AL, ML, and NBL) using qRT-PCR with the transcription of GAPDH gene as a control.

Comparison between the same stage and same concentration of P4

The results of the comparative expression of the *Ts-MAPRC2* gene in the F-AL stage at different concentration of P4 (P300 ng/mL, P100 ng/mL, P30 ng/mL, P10 ng/mL, and P3 ng/mL) with control (only RPMI) and control vehicle ethanol (EtOH-RPMI) are presented in Fig. 6A. All concentrations of P4 (P300 ng/mL, P100 ng/mL, P30 ng/mL, P10 ng/mL, and P3 ng/mL) showed up-regulation of the *Ts*-MAPRC2 gene as compared to control (only RPMI) and control vehicle ethanol (EtOH-RPMI); in contrast, the P30 showed high up-regulation expression of the *Ts*-MAPRC2 gene compared to all other concentrations with their both controls (only RPMI and EtOH-RPMI) ($P \leq 0.05$). Figure 6B showed the comparative expression of *Ts*-MAPRC2 gene in the M-AL stage of different concentration of P4 (P300 ng/mL, P100 ng/mL, P30 ng/mL, and P10 ng/mL, P3 ng/mL) with control (only RPMI) and control vehicle ethanol (EtOH-RPMI). All concentrations of P4 (P300 ng/mL, P100 ng/mL, P30 ng/mL, P10 ng/mL, and P3 ng/mL) showed up-regulation of the *Ts*-MAPRC2 gene relative to control (only RPMI) and control vehicle ethanol (EtOH-RPMI), but expression decreased in descending order from high concentration (P300 ng/mL) to low concentration (P3 ng/mL) compared with both controls (only RPMI and EtOH-RPMI) ($P \leq 0.05$). comparative expression of the *Ts-MAPRC2* gene in the ML stage at different concentration of P4 (P300 ng/mL, P100 ng/mL, P30 ng/mL, P10 ng/mL, and P3 ng/mL) with control (only RPMI) and control vehicle ethanol (EtOH-RPMI) are presented in the Fig. 6C. In the ML stage, all treatment concentrations showed up-regulation of the *Ts*-MAPRC2 gene, but P300 and P30 showed more upregulation compared with both controls (only RPMI and EtOH-RPMI) ($P \leq 0.01$). Figure 7D shows the relative expression of the *Ts-MAPRC2* gene in the NBL stage

at different concentration of P4 (P300 ng/mL, P100 ng/mL, P30 ng/mL, P10 ng/mL, and P3 ng/mL) with control (only RPMI) and control vehicle ethanol (EtOH-RPMI). All concentrations of P4 (P300 ng/mL, P100 ng/mL, P30 ng/mL, P10 ng/mL, and P3 ng/mL) showed down-regulation compared with control (only RPMI), but here control vehicle ethanol (EtOH-RPMI) also showed down-regulation.

Comparison among the same stage and same concentration of mifepristone (RU486)

Relative expression of the *Ts*-MAPRC2 gene in the F-AL stage at different concentrations of mifepristone (M300 ng/mL, M100 ng/mL, and M30 ng/mL) with control (only RPMI) and control vehicle ethanol (EtOH-RPMI) are presented in Fig. 7A. All concentrations showed up-regulation of the *Ts*-MAPRC2 gene as compared with control (only RPMI) and control vehicle ethanol (EtOH-RPMI), but M100 (ng/mL) showed high up-regulation expression of the *Ts*-MAPRC2 gene compared to all other treatment concentrations with their both controls (only RPMI and EtOH-RPMI) ($P \leq 0.05$). Figure 7B shows the comparative expression of the *Ts*-MAPRC2 gene in the M-AL stage at different concentrations of mifepristone (M300 ng/mL, M100 ng/mL, and M30 ng/mL) with control (only RPMI) and control vehicle ethanol (EtOH-RPMI). Here only M100 (ng/mL) showed up-regulation of the *Ts*-MAPRC2 gene as compared with control (only RPMI) and control vehicle ethanol (EtOH-RPMI); however, the M300 (ng/mL) showed expression same as control vehicle ethanol (EtOH-RPMI), and the M30 (ng/mL) showed expression same as control (only RPMI) ($P \leq 0.05$). Figure 7C shows the comparative expression of the *Ts*-MAPRC2 gene in the ML stage at different concentrations of mifepristone (M300 ng/mL, M100 ng/mL, and M30 ng/mL) with control (only RPMI) and control vehicle ethanol (EtOH-RPMI). In the ML stage, M100 (ng/mL) showed up-regulation of the *Ts*-MAPRC2 gene as compared with control (only RPMI) and control vehicle ethanol (EtOH-RPMI) but M300 (ng/mL) and M30 (ng/mL) showed almost the same expression compared with both controls (only RPMI and EtOH-RPMI) ($P \leq 0.05$). Figure 8D shows the relative expression of the *Ts*-MAPRC2 gene in the NBL stage at different concentrations of mifepristone (M300 ng/mL, M100 ng/mL, and M30 ng/mL) with control (only RPMI) and control vehicle ethanol (EtOH-RPMI). In the NBL stage, the expression decreased in descending order from high concentration (M300 ng/mL) to low concentration (M100 ng/mL) compared with both controls (only RPMI and EtOH-RPMI) ($P \leq 0.05$). But M30 (ng/mL) showed downregulation respectively ($P \leq 0.05$).

Comparison among the P4 and RU486 at F-AL stage by using P30 and M30 as controls

Relative expression analysis of the *Ts*-MAPRC2 gene in the F-AL stage at different concentrations of P4 (P300 ng/mL, P100 ng/mL, P30 ng/mL, P10 ng/mL, and P3 ng/mL) with M30 ng/mL as control are presented in Fig. 8A. P100 ng/mL, P30 ng/mL, and P10 ng/mL showed up-regulation of the *Ts*-MAPRC2 gene while P300 ng/mL, and P3 ng/mL showed down-regulation as compared with M30 ng/mL as control. Figure 8B shows the comparison expression of the *Ts*-MAPRC2 gene in the F-AL (Female adult worm) stage at different concentrations of mifepristone (M300 ng/mL, M100 ng/mL, and M30 ng/mL) with P30 ng/ml as control. Here all concentrations of mifepristone (M300 ng/mL, M100 ng/mL, and M30 ng/mL) showed down-regulation compared with P30 ng/mL as control ($P \leq 0.05$).

In-Vitro phenotypic effect of P4, RU486, and rTs-MAPRC2-Ab on F-AL and ML stages

In-vitro phenotypic effect of P30 ng/mL, M100 ng/mL, and *rTs-MAPRC2-Ab* on the F-AL stage revealed that observed pregnancy and abortion to produce NBL parasites (Fig. 9A**)**. M100 (ng/mL) and *rTs-MAPRC2-Ab* showed more aborted NBL parasites as compared to controls (only RPMI and EtOH-RPMI), while P30 ng/mL prolonged pregnancy period and delays abortion to NBL compared with controls (only RPMI and EtOH-RPMI). Figure 9B shows the *in-vitro* phenotypic effect of P30 ng/mL, M100 ng/mL, and *rTs-MAPRC2-Ab* on the ML stage. Here, the M100 has more ecdysis (molting process) and motility compared with P30 (ng/mL) concentration, *rTs-MAPRC2-Ab*, and controls (only RPMI and EtOH-RPMI). While *rTs-MAPRC2-Ab* also has high motility compared to P30 ng/mL, and both controls (only RPMI and EtOH-RPMI).

Phenotypic effect and relative mRNA expression of mifepristone on F-AL stage by in-vivo

In-vivo phenotypic effect of mifepristone (M100 ng/mL) on F-AL (female adult worm) are presented in Fig. 10A. Comparison of the early pregnancy stage of F-AL among M100 ng/mL, EtOH (Olive oil-ethanol) with the simple control mice groups. The simple control group and EtOH (Olive oil-ethanol) showed normal pregnancy at 6 dpi (days post-infection), but the F-AL of the M100 ng/mL treated mice group did not show pregnancy and only see embryos inside of the F-AL body. *In-vivo* study shows the relative mRNA expression of *Ts-MAPRC2* at F-AL phase of *T. spiralis* among three groups such as M100 ng/mL, EtOH (Olive oil-ethanol) with the simple control (Fig. 10B**)**. M100 ng/mL showed up-regulation of the *Ts*-MAPRC2 gene as compared with EtOH (Olive oil-ethanol) with the simple control ($P \leq 0.05$).

Discussions

Progesterone receptor membrane component 1&2 *(PGRMC-1& PGRMC-2)* belongs to the same family of MAPR (membrane-associated progesterone receptor) proteins (36, 37), and *PGRMC-1* protein was collected first from smooth muscle of porcine with 28 kDa (38, 39). It is located in the cell membrane, endoplasmic reticulum (ER), Golgi apparatus, and consisted of a small extracellular N-terminal cytoplasmic domain and a single transmembrane domain (38–43). It also contains the sequences that bind with steroids, cytochrome-b5, and three Src similar domains that participated in ligand-dependent signal-transduction (39). *PGRMC-1* expressed in many mammals (e.g., human, rodents, monkey, pigs, and cattle) reproductive tissues, and it involved in many functions i.e., steroidogenesis, cholesterol metabolism regulation, oocyte maturation, and myometrial contractility, etc.(21, 39, 41, 43–47). It is also involved in the *in-vitro* existence of regular and cancerous ovarian cells (48). PRMC-2 resembled PGRMC-1, but the sequence domains of these two proteins differ in the transmembrane and N-terminal stages, showing a potential capacity of both receptors' interactions (49). *PGRMC-2* also showed mRNA expression in the endometrium wall of monkeys and mice as well as in the myometrium, and endometrium of cattle's oviduct (50, 51). Prior studies noted that in humans women showed upregulated expression in choriodecidual tissue, especially in 35 weeks of gestation (pre-term labor) and breast adenocarcinoma (21, 41, 50–54). Likewise in helminths, a few studies reported PGRMC receptors, progestin-induced protein, p48 progesterone-receptor-associated protein, and small androgen receptor-interacting proteins that were found in *S. japonicum* (24, 25). The pleiotropic role of membrane-associated progesterone receptor component-2 *(MAPRC2)* in the

biology of the reproductive system of *T. spiralis* suggest that it can be a new target for drug and vaccine development against *Trichinellosis*.

To this end, in this study we cloned, expressed the *MAPRC2* gene in *T. spiralis*, and evaluated its expression levels with P4 (agonist) and RU486 (antagonist) hormones. Cytochrome-b5 heam/steroid binding domain present inside the *Ts-MAPRC2* protein (6-75aa), and calculated with BLAST. An online bioinformatics tool (TMHMM) shows that there was not any transmembrane found in the cloned sequence (225-705bp). Notably, the percent matrix index (Fig. S2C) displayed more resemblance between the *Ts-MAPRC2* and strains of other *Trichinella* species. Interestingly, we magnificently cloned the *Ts-MAPRC2* gene, and its protein expression was confirmed *via* SDS-PAGE. The host immune system recognized *rTs-MAPRC2* protein confirmed by immunoblot assay suggested that the recombinant protein has the compatible antigenic features (Figs. 2 & 3). Moreover, a binding capacity *rTs-MAPRC2* protein with a progesterone antibody (PROG-Ab) was determined by Sandwich ELISA kit (Fig. 4). This finding complements the idea that *rTs-MAPRC2* protein has a specific steroid-binding domain that might arbitrate the P4 effect in the reproductive process of *T. spiralis*. Likewise, *PGRMC-2* detected mainly in reproductive parts of mammals (ovary, endometrium, placenta) associated with multiple cellular functions (differentiation, proliferation, and maturation), and also identified in epithelial cells, granulocytes, mast cells, lymphocytes, and macrophages (36, 55–57).

Furthermore, we examined the localization of the *MAPRC2* gene in *T. spiralis*, immunofluorescence staining at all developmental stages that confirmed the presence of this gene throughout all the developmental stages (Fig. 5). Existing studies also performed the immunofluorescence assay to examine membrane-binding progesterone protein in the *T. solium* cysticerci (23). likewise, many studies reported that progesterone also showed immunomodulatory response during pregnancy diverged towards the Th2 type response to activate the innate immune system (14, 58). Basically, in females, Th2 type immune response increased the interleukins production (IL-4,-5,-6,10) (57). Hence, in pregnant rats, resistance against *T. spiralis* has been reported due to a high progesterone level compared with non-pregnant rats. Also, *in-vitro* trials showed that pregnant rat sera could interrupt the death of the NBL stage of *T. spiralis* (59). From these, either stimulatory or inhibitory function of progesterone affects the immune response, based on the concentration, steroid exposure time, and type of cell studied (60). Therefore, based on the murine model, both sexes were studied against *Trichinella* infections, and the result showed that males mice are more susceptible to infection than female mice (61–63). Further, parasite burden interestingly increased in ovariectomized female rats compared with intact females (64). Mifepristone (RU486) worked as antagonists against glucocorticoid receptor (GR) and progesterone receptor (PR) and through anticancer and abortifacient actions (19). Mifepristone, one of the first medications approved for surgical abortion, is often used to end an early or mid-term pregnancy (26).

The *in-vitro* study shows relative mRNA expression of *Ts-MAPRC2* gene at all stages (ML, F-AL, and M-AL, NBL) treated with three different concentration of progesterone (P300 ng/mL, P100 ng/mL, P30 ng/mL, P10 ng/mL, and P3 ng/mL), mifepristone (M300 ng/mL, M100 ng/mL, M30 ng/mL), and their controls (ETH-RPMI & only RPMI) ($P \leq 0.05$). Figure 6 shows the comparison expression of the *Ts*-MAPRC2 gene in

all stages (F-AL, M-AL, ML, and NBL) at a changed concentration of P4 (P300 ng/mL, P100 ng/mL, P30 ng/mL, P10 ng/mL, P3 ng/mL) with both controls ($P \leq 0.05$). All concentrations of P4 (P300 ng/mL, P100 ng/mL, P30 ng/mL, P10 ng/mL, and P3 ng/mL) showed up-regulation of the *Ts*-MAPRC2 gene in three stages (F-AL, M-AL, and ML) but NBL showed down-regulation as compared with both controls ($P \leq 0.05$). In the F-AL stage, P30 showed high up-regulation expression of the *Ts-MAPRC2* gene compared to all other concentrations with both controls ($P \leq 0.05$). From these results, one can argue that P300 ng/mL concentration of P4 levels up to a certain limit moves toward downregulation; this strongly emphasizes the maximum levels of progesterone range 100–200 ng/mL in rats affect the death of new borne larvae in *T. spiralis* (17).

Figure 7 shows the comparison expression of the *Ts-MAPRC2* gene in all stages (F-AL, M-AL, ML, and NBL) at different doses of mifepristone (M300 ng/mL, M100 ng/mL, and M30 ng/mL) with controls (only RPMI and EtOH-RPMI). All concentrations of (M300 ng/mL, M100 ng/mL, and M30 ng/mL) showed up-regulation expression in F-AL, M-AL and ML stages, but in the NBL stage only M300 ng/mL, M100 ng/mL showed up-regulation expression compared by controls ($P \leq 0.05$). In the F-AL stage, only M100 ng/mL showed high up-regulation expression of the *Ts-MAPRC2* gene that confirms this concentration is good as antagonists against progesterone receptor (PR) as well as abortifacient activities. Further, we confirmed through a cross-comparison expression of the *Ts*-MAPRC2 gene among the P4 and RU486 at the F-AL stage by P30 ng/mL and M30ng/mL used as controls. Figure 8A compared the expression of the *Ts*-MAPRC2 gene in the F-AL stage in all concentrations of P4 (P300 ng/mL, P100 ng/mL, P30 ng/mL, P10 ng/mL, and P3 ng/mL) with M30 ng/mL as control. The P100 ng/mL, P30 ng/mL, and P10 ng/mL showed upregulation, while P300 ng/mL, P3ng/mL showed downregulation with M30 ng/mL as control but noted that P30 ng/mL still shows high up-regulation. Figure 8B compares the expression of the *Ts*-MAPRC2 gene in the F-AL stage at different concentrations of mifepristone (M300 ng/mL, M100 ng/mL, and M30 ng/mL) with P30 ng/mL as control. All concentration of mifepristone (M300 ng/mL, M100 ng/mL, M30 ng/mL) showed down-regulation compared to P30 ng/mL as control ($P \leq 0.05$). Finally, we concluded that the progesterone (P4) upregulate the *Ts*-MAPRC2 gene at a low concentration of P30 ng/mL but downregulated the *Ts*-MAPRC2 gene at P300 ng/mL, in the F-AL stage compared with M30 ng/mL positive control, this corroborated with the findings of (17) indicating more resistance against *T. spiralis* due to a high progesterone level compared with virgin rats. Also, *in-vitro* trials showed pregnant rat sera could interrupt newborn larvae (NBL) death of *T. spiralis* (59). Although, RU486 (antagonist) downregulated the *Ts*-MAPRC2 gene at all concentrations (M300 ng/mL, M100 ng/mL, and M30 ng/mL) compared with P30 ng/mL as a positive control that claims the mifepristone (RU486) function as antagonists against progesterone receptor (PR) and abortifacient activities (19).

Based on this analysis, we subsequently examined the phenotypic appearance of pregnant F-AL among P30 (ng/mL), M100(ng/mL), and *rTs-MAPRC2-Ab* with controls (only RPMI and EtOH-RPMI) by *in-vitro*. M100 ng/mL and *rTs-MAPRC2-Ab* showed more aborted NBL parasites as compared to controls while P30 ng/mL prolong pregnancy and delays in abortion to produce NBL compared with control groups (only RPMI and EtOH-RPMI) (see Fig. 9A), these findings are in line with the findings of (17, 19). Further, Fig. 9B shows the comparison of the phenotypic appearance of muscle larvae (ML) among P30 (ng/mL), M100

ng/mL, and *rTs-MAPRC2-Ab* with both controls (only RPMI and EtOH-RPMI) to observe ecdysis (molting process) and motility. M100 ng/mL has more ecdysis (molting process) and motility compared with P30 ng/mL concentration, *rTs-MAPRC2-Ab*, and controls (only RPMI and EtOH-RPMI). While *rTs-MAPRC2-Ab* has high motility compared to P30 ng/mL and both controls (only RPMI and EtOH-RPMI) correspondingly supported by the finding of (65). Figure 10 shows the *in-vivo* study of phenotypic effect and relative mRNA expression of M100 ng/mL on the F-AL stage. The simple control group and EtOH (Olive oil-ethanol) shows normal pregnancy at 6 dpi (days post-infection) but F-AL of M100 ng/mL treated mice grouped did not show pregnancy and only see embryos inside of the F-AL body. M100 ng/mL showed up-regulation of the *Ts-MAPRC2* gene as compared with EtOH (Olive oil-ethanol) and the simple control ($P \leq 0.05$)(Fig. 10B), these findings are amply supported by (19, 65).

Conclusions

To conclude, the M100 ng/mL were resulting in downregulation of the *Ts-MAPRC2* gene, which in turn leads to abortion *in vivo* as well as *in vitro* in F-AL. However, the P30 ng/mL restrains the pregnancy in the early stage of F-AL. P30 ng/mL showed up-regulation of the target gene and had a positive effect on the pregnancy as compared to P300 ng/mL. To the best of our knowledge, this is the first study regarding cloning, expression of the *rTs-MAPRC2* gene, and its expression level with sex hormones (P4 & RU486) at all developmental stages (ML, F-AL, and M-AL, NBL) as well as *in-vitro* and *in-vivo* phenotypic effects in F-AL stage of *T. spiralis*. This opens a new horizon about antihormone (P4 & RU486) drug design, and vaccine therapy of recombinant (*rTs-MAPRC2*) protein with their combined effect to control *Trichinellosis*.

Abbreviations

AL: Adult worms; NBL: new borne larvae; ML: muscle larvae; F-AL: female adult worm; M-AL: male adult worm; PR: progesterone receptor; *Ts-MAPRC2: Trichinella spiralis* membrane-associated progesterone receptor component-2; P4: Progesterone; RU486: Mifepristone; EtOH: Olive oil-ethanol

Declarations

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Authors' contributions

YRF directed the project and participated in the coordination and management of the study. MTA performed the laboratory experiments and wrote the manuscript. SJ, ZQY, ZW, ZY, LM assisted on the laboratory tests on *Trichinella spiralis* species. SAL, MH, MWH, analyzed the data, and HA, MWH, provided some ideas for the experimental design. LXR, SXK, and XLX provided new analytical reagents and tools. All authors read and approved the final manuscript.

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Availability of data and materials

All data generated or analyzed during this study are included within the article and its additional information file.

Ethics approval and consent to participate

Regarding the animals included in our research, this study followed the guidelines of the Animal Ethics Committee, Nanjing Agricultural University, China. The approval ID was SCXK 2008-0004.

Consent for publication

Written informed consent was obtained from the participants for the publication of this paper.

Conflicts of Interest

The authors declare no conflict of interest

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Figures

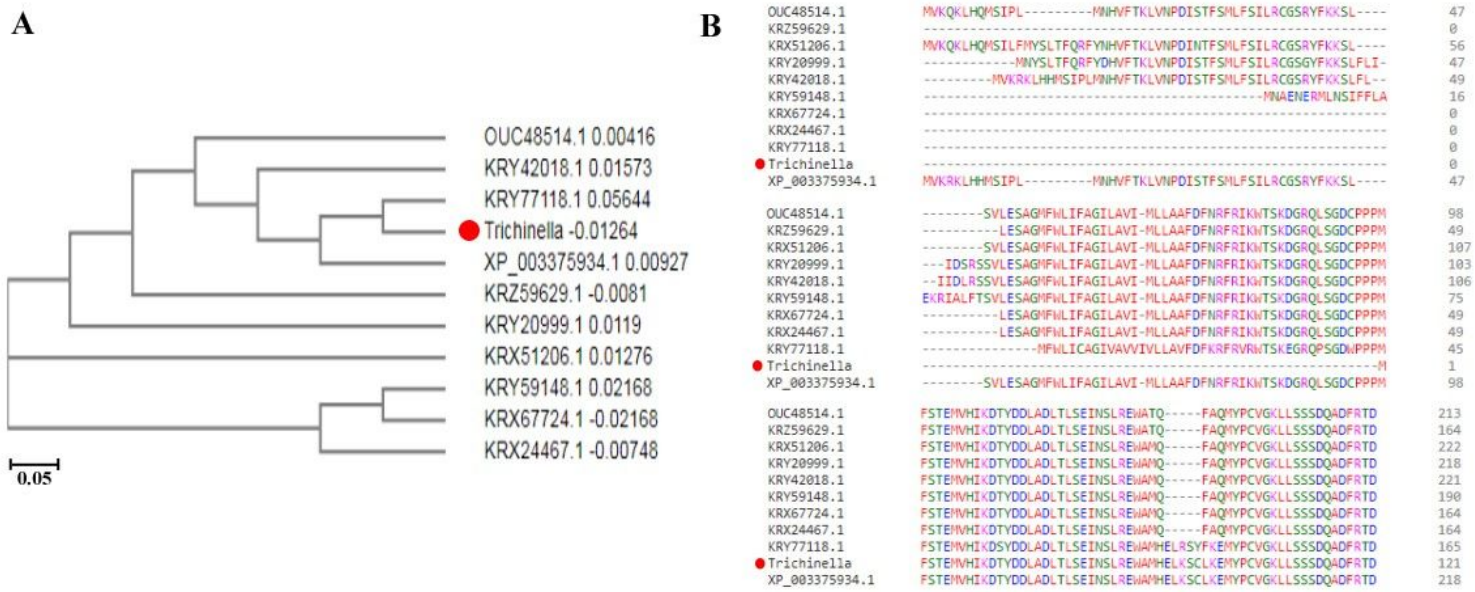

Figure 1

Phylogenetic tree and multiple sequence alignment. (A) A phylogenetic tree of Ts-MAPRC2 and homologs with other species. (B) Multiple Sequence Alignment of Ts-MAPRC2 by Clustal Omega with other trichinella species. Different colors show the amino acid identity, and gaps represent the sequence with other trichinella species, respectively.

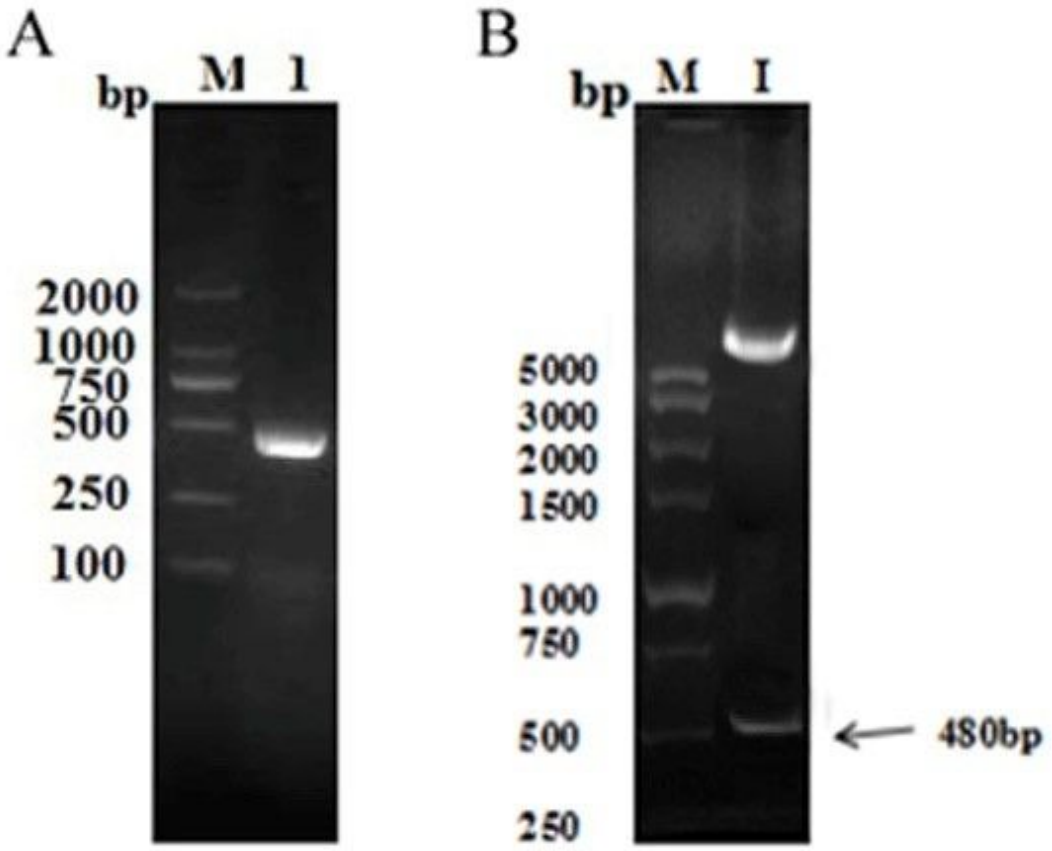

Figure 2

Cloning of Ts-MAPRC2 gene and identification of recombinant plasmid (pET-32a (+) Ts-MAPRC2. Lane M represents DNA marker (A) Lane 1; Ts-MAPRC2 gene amplified by PCR (B) Lane 1; Digested recombinant plasmid {(pET-32a (+)} Ts-MAPRC2 with EcoR1 and Hind III.

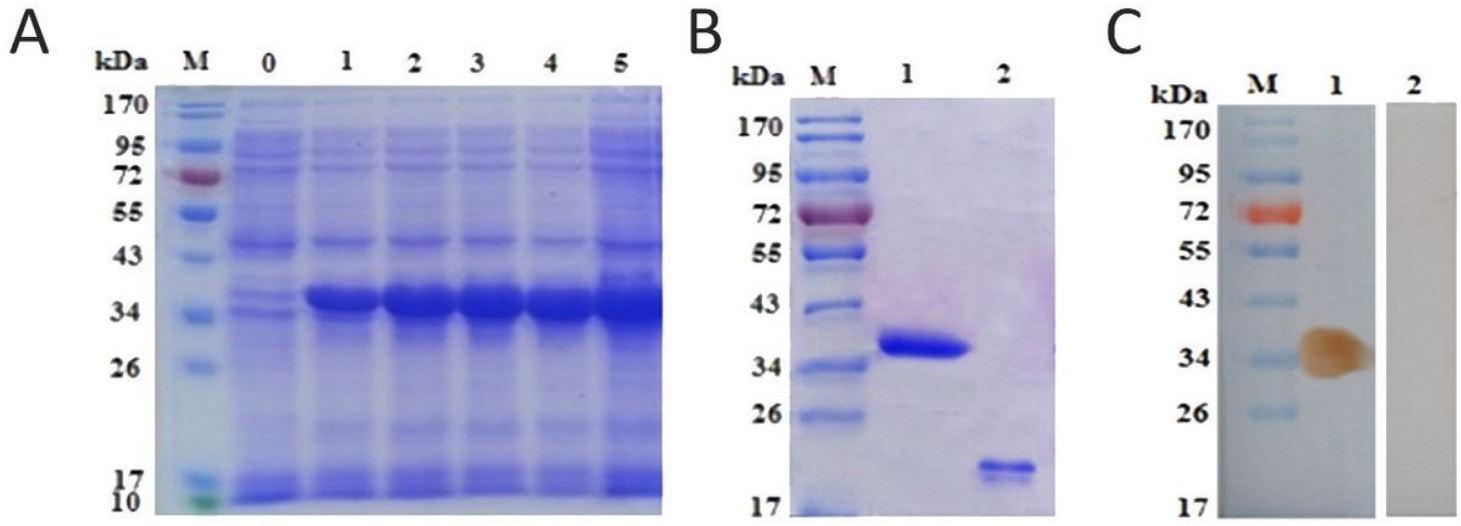

Figure 3

Expression and purification of rTs-MAPRC2. Lane M: Standard molecular weight of protein marker Lane 0: Recombinant expression vector prior IPTG (isopropyl-ß-d-thiogalactopyranoside) induction. (A) Lane 1-5: Expression of rTs-MAPRC2 protein after IPTG induction at different time points. (B) Lane 1: Purified expression of the rTs-MAPRC2 protein. Lane 2: Expression of pET-32a protein resolved on SDS-PAGE. (C) M: Standard marker molecular weight. Lane 1: Purified rTs-MAPRC2 protein was shifted to the membrane and probed with SD rat serum immunized by rTs-MAPRC2 protein. Lane 2: Membrane probed by normal rat serum as control.

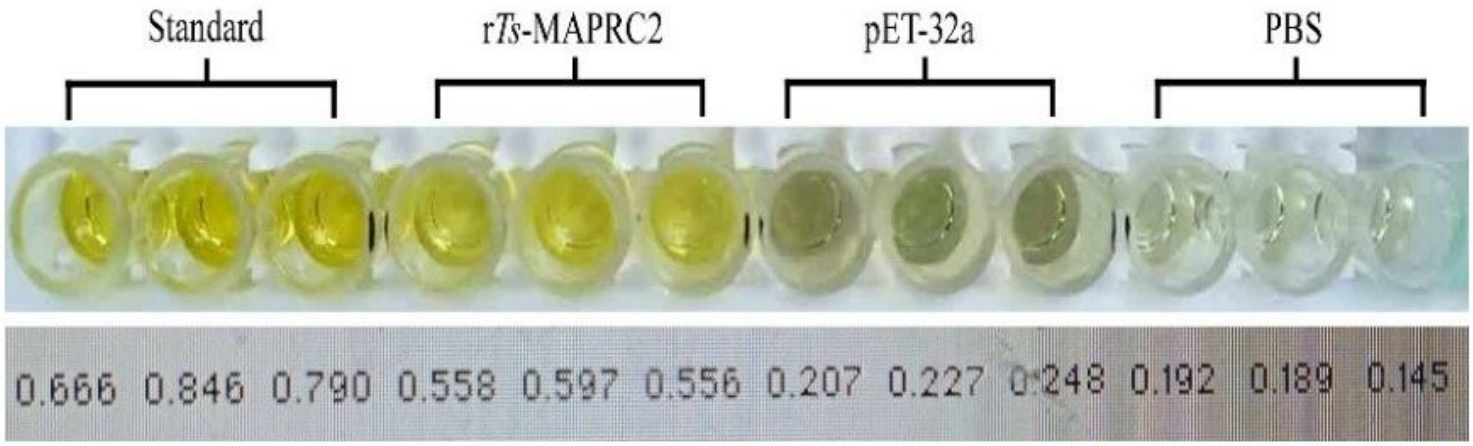

Figure 4

The binding ability of rTs-MAPRC2 protein compared to pET-32a and PBS as control by using a (PROG-Ab) Sandwich ELISA kit (OD450). The data were shown in three independent trials (n=3).

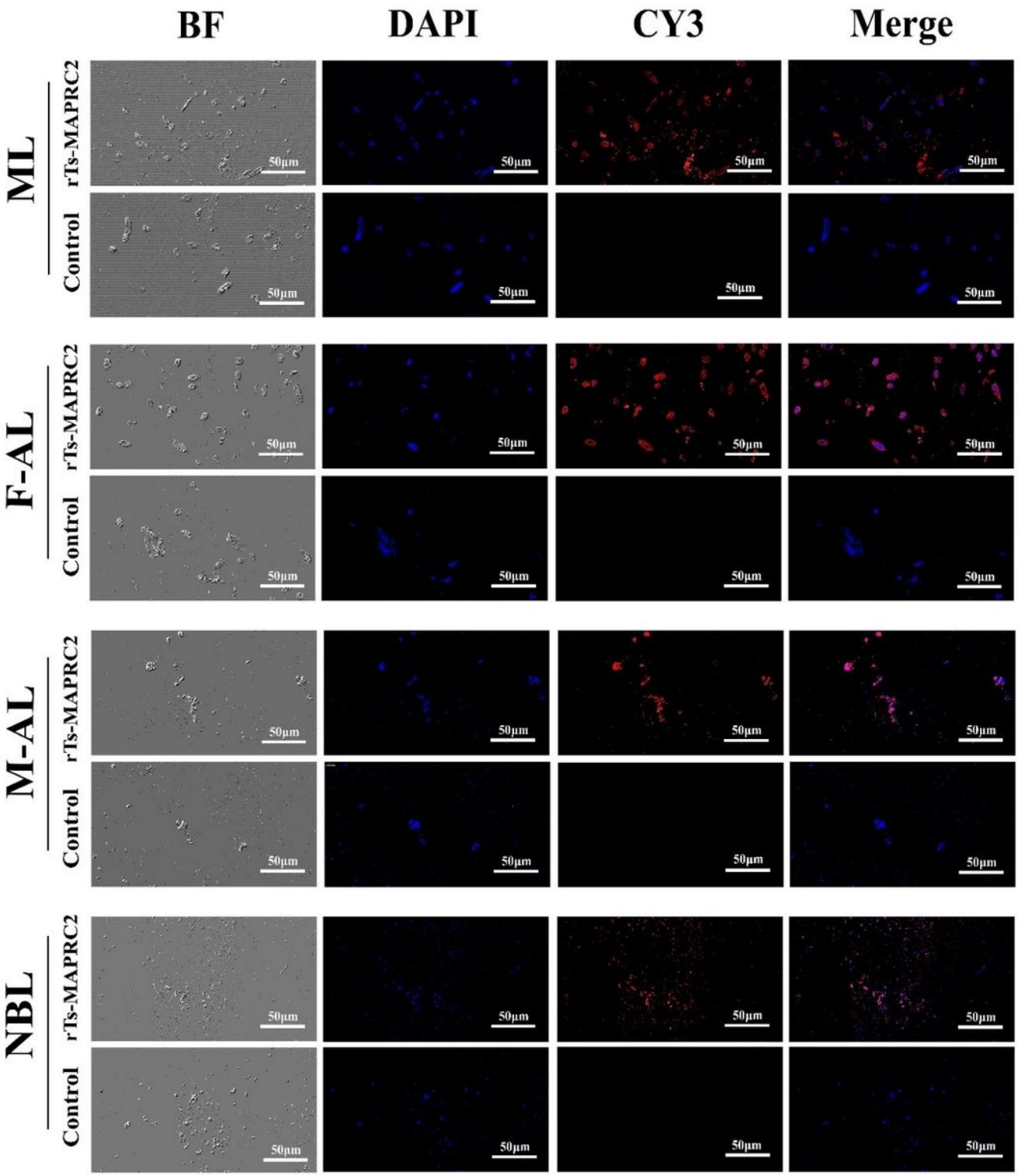

Figure 5

Immunolocalization of Ts-MAPRC2 at different developmental stages of T. spiralis. Cross-section of Intact worms of all stages (F-AL, M-AL, ML, and NBL) were studied using IFA with anti-rTs-MAPR sera followed by BF (Bright field), DAPI, staining with Cy3-conjugated secondary antibody, and Merge. ML and F-AL stages showed high Ts-MAPR gene localization as compared to M-AL and NBL stages. Moreover, F-AL showed

high immunolocalization compared to M-AL. No fluorescence was observed in control of all the worm's developmental stages with a scale bar of 50 μm.

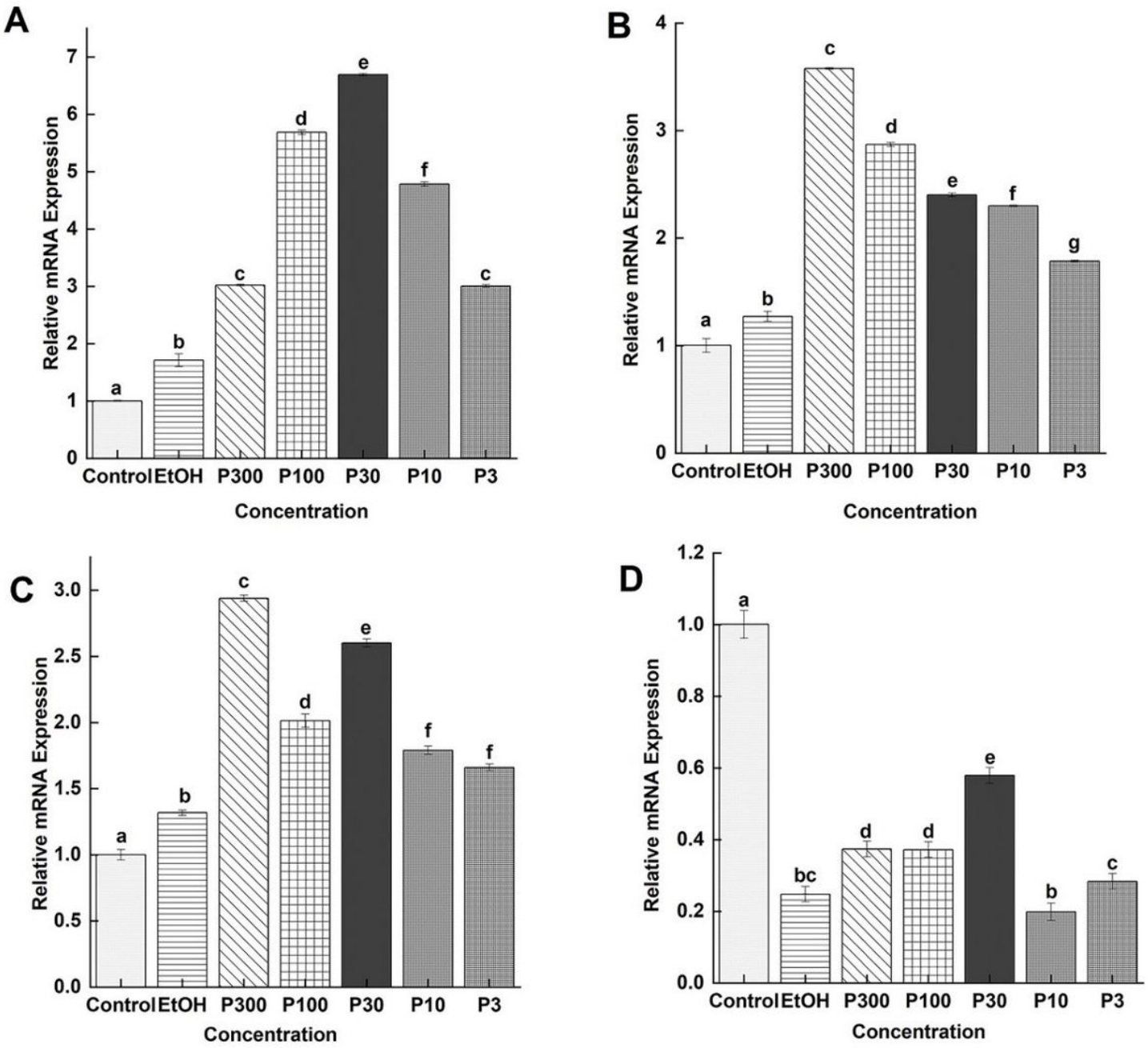

Figure 6

Relative mRNA levels of Ts-MAPRC2 at various developmental phases of T. spiralis. The Comparison between the different concentration of P4 (P300 ng/mL, P100 ng/mL, P30 ng/mL, P10 ng/mL, P3 ng/mL) with control (only RPMI) and control vehicle ethanol (EtOH-RPMI) among the same developmental stages (A) F-AL (B) M-AL (C) ML (D) NBL of Trichinella spiralis. Statistical data were presented as mean ± SD (n=3). $P \leq 0.05$, $P \leq 0.01$ were considered significant. The same letters mean non-significant and the different letters mean significant.

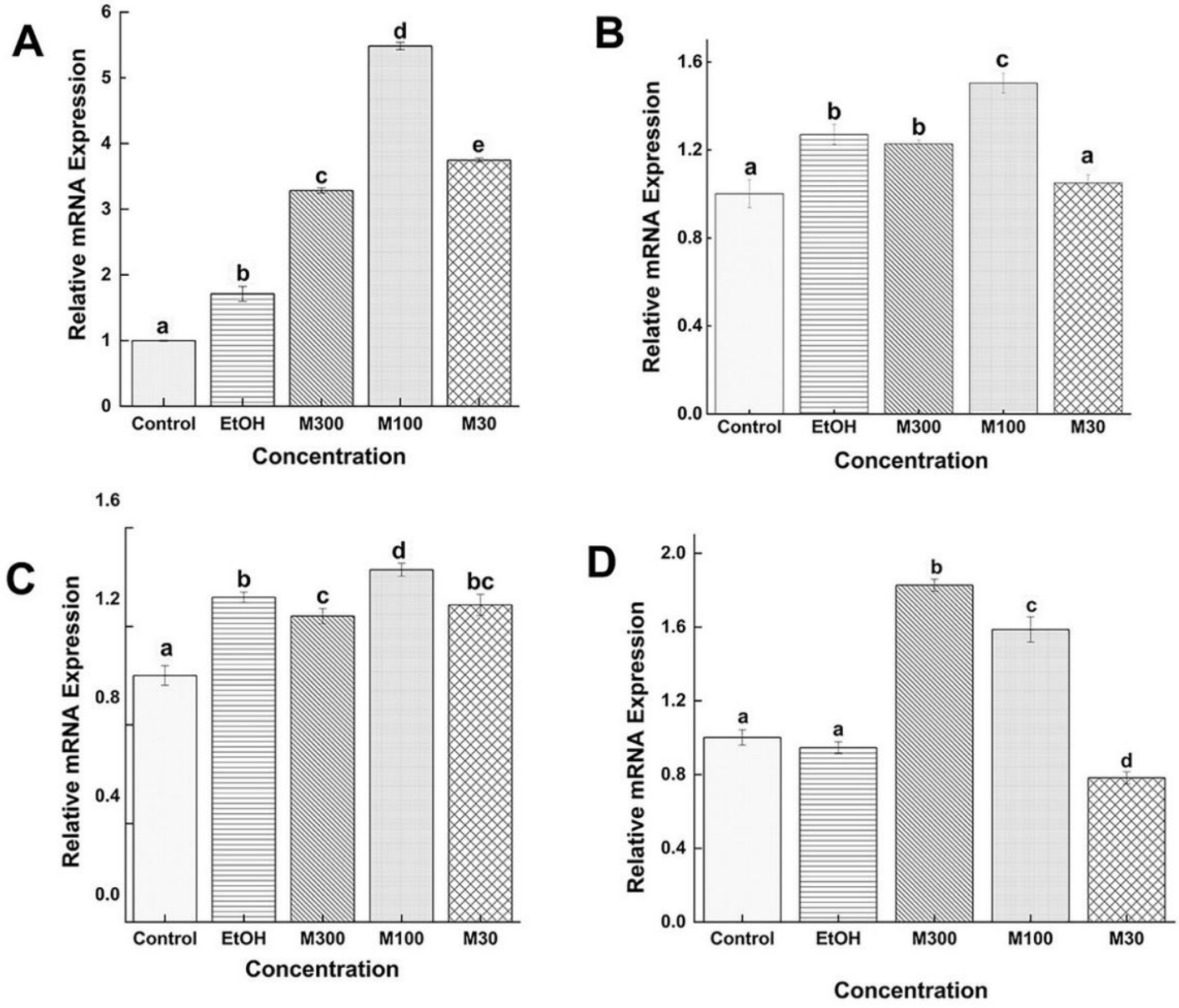

Figure 7

Relative mRNA levels of Ts-MAPRC2 at various developmental phases of T. spiralis. The Comparison between the different concentration of mifepristone (M300 ng/mL, M100 ng/mL, M30 ng/mL) with control (only RPMI) and control vehicle ethanol (EtOH-RPMI) among the same developmental stages (A) F-AL (B) M-AL (C) ML (D) NBL of Trichinella spiralis. Statistical data were presented as mean ± SD (n=3). $P \leq 0.05$ were considered significant. The same letters mean non-significant and the different letters mean significant.

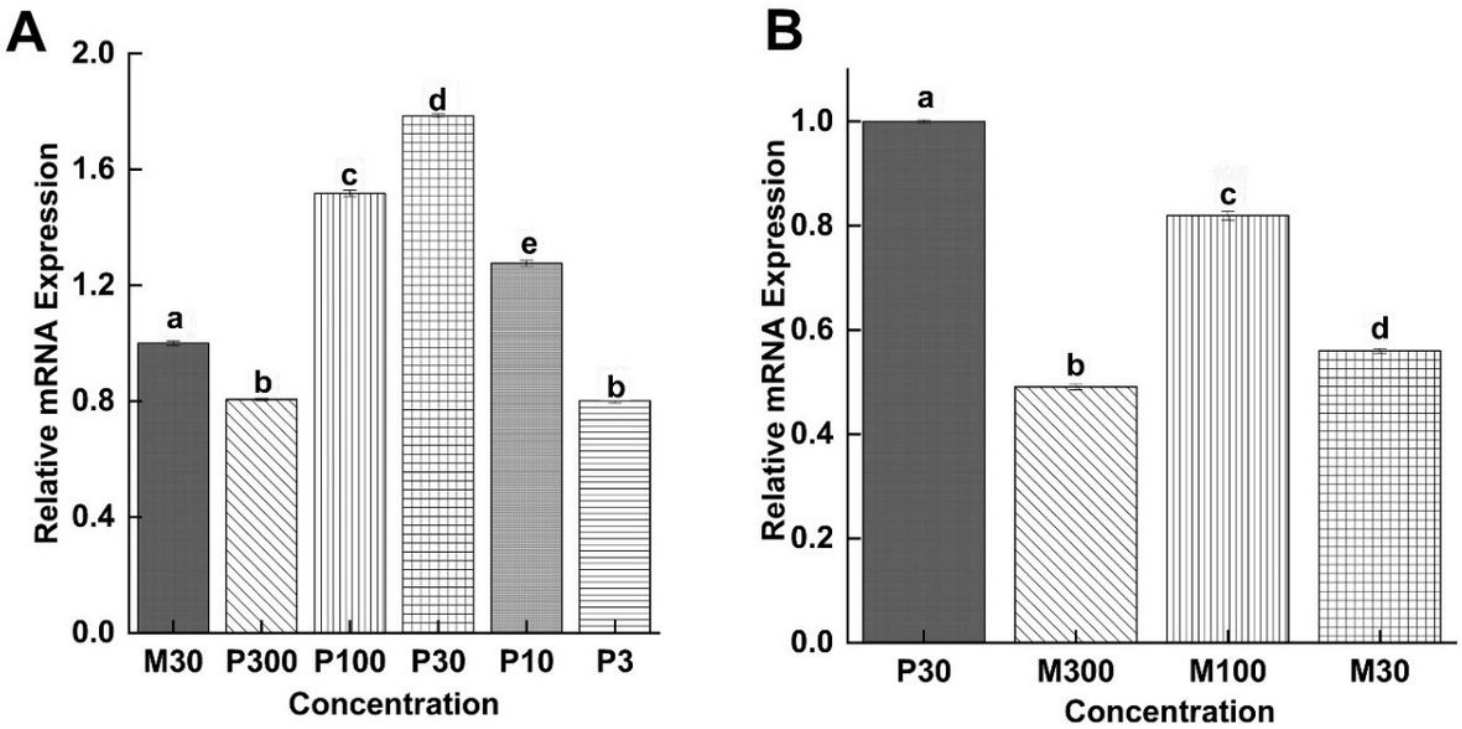

Figure 8

Relative mRNA expression of Ts-MAPRC2 at F-AL (Female adult worm) phase of T. spiralis. (A) Comparison between the different concentration of P4 (P300 ng/mL, P100 ng/mL, P30 ng/mL, P10 ng/mL, P3 ng/mL) with M30 ng/mL as control. (B) Comparison between the different concentration of mifepristone (M300 ng/mL, M100 ng/mL, M30 ng/mL) with P30 ng/mL as control. Statistical data were presented as mean ± SD (n=3). $P \leq 0.05$ were considered significant. The same latter's means non-significant and different letters mean significant.

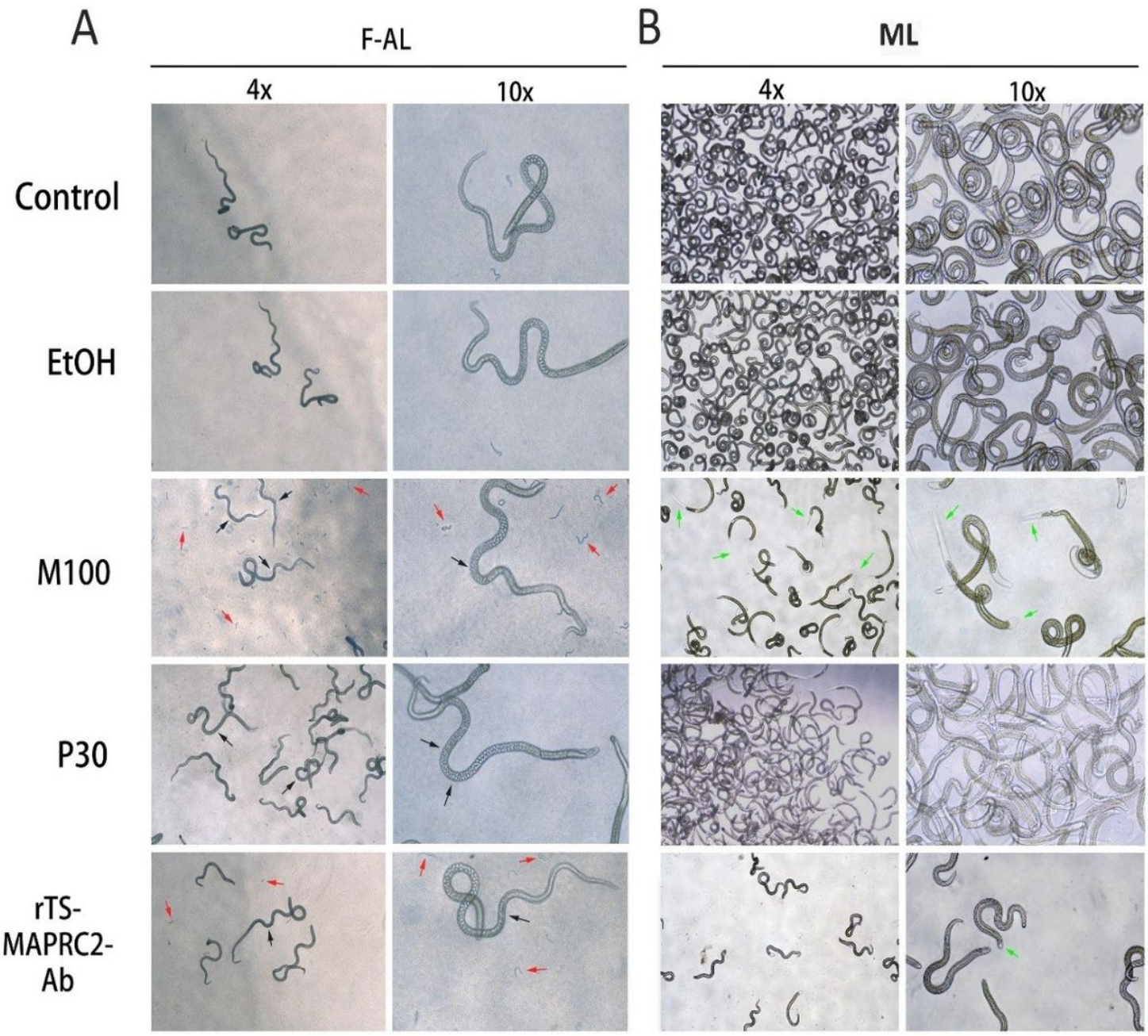

Figure 9

The in-Vitro phenotypic effect of P30 (ng/mL), M100(ng/mL), and rTs-MAPRC2-Ab on F-AL (female adult worm) and ML (muscle larvae) stages were observed. (A) Compared the phenotypic appearance of pregnant female adult worm (F-AL) among P30 (ng/mL), M100(ng/mL), and rTs-MAPRC2-Ab with both controls (only RPMI and EtOH-RPMI). Black arrows presented a female pregnancy site while red arrows present new borne larvae (NBL) at objective 4x and 10x. (B) Compared the phenotype appearance of muscle larvae (ML) among P30 (ng/mL), M100(ng/mL), and rTs-MAPRC2-Ab with both controls (only RPMI and EtOH-RPMI) to observed ecdysis (molting process is shown by green arrow) and motility at objective 4x and 10x.

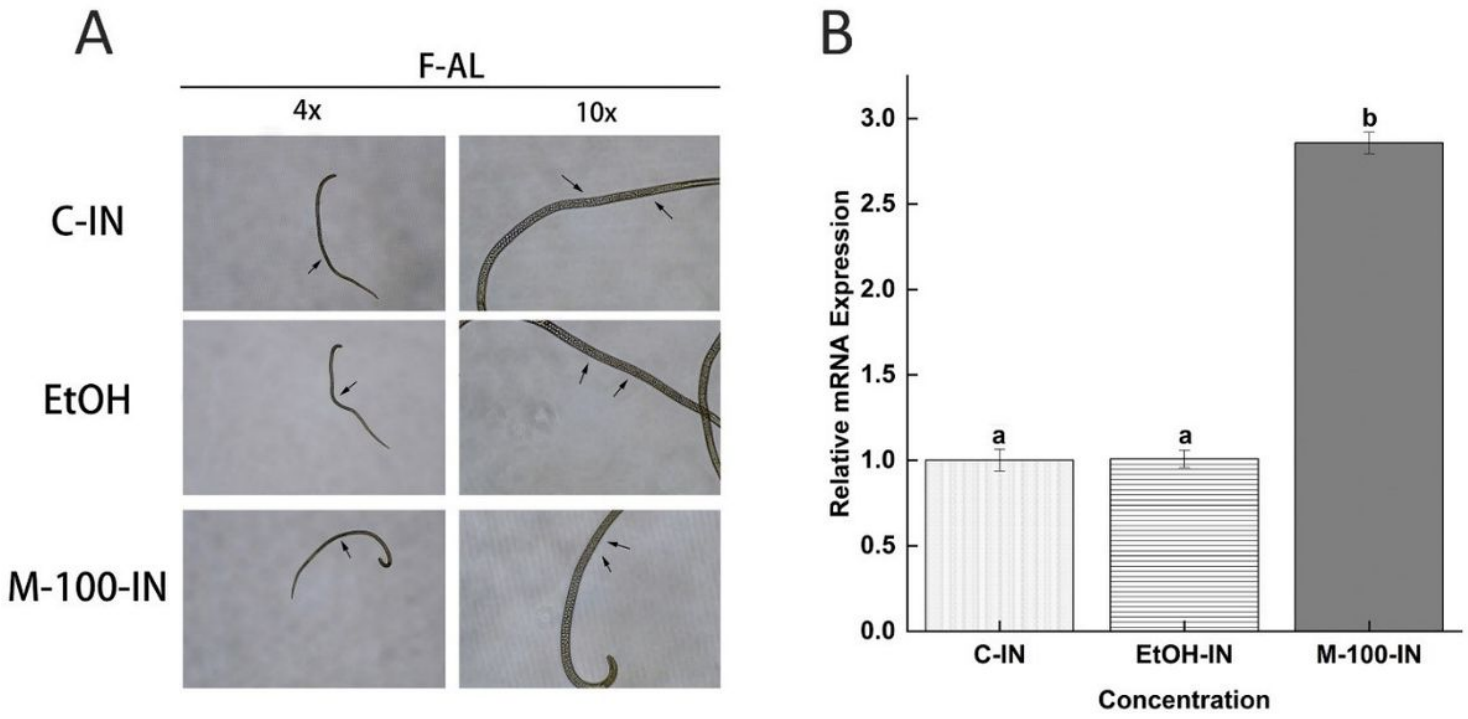

Figure 10

The in-vivo phenotypic effect and relative mRNA expression of mifepristone (M100 ng/mL) on F-AL (female adult worm) were observed. (A) Compared the phenotypic appearance of early pregnancy stage of female adult worm (F-AL) among M100(ng/mL) with control and adjuvants EtOH (Olive oil-ethanol) at objective 4x and 10x. (B) Relative mRNA expression of Ts-MAPRC2 at F-AL (Female adult worm) phase of T. spiralis by in-vivo study. Here a comparison between M100 (ng/mL) with a simple control group and adjuvants EtOH (Olive oil-ethanol) group. Statistical data were presented as mean ± SD (n=3). $P \leq 0.05$ were considered significant. The same letters mean non-significant and different letters mean significant.

Supplementary Files

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- SupplementaryFileS1.docx