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Research Article

Potential of nanoemulsion of spiramycin in alleviating histological and embryonic changes in Swiss albino mice infected with congenital toxoplasmosis

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Abstract
Congenital toxoplasmosis can lead to harm to tissues and necessitate suitable treatments for better outcomes in affected cases.The present research assesses the effectiveness of spiramycin nanoemulsion combined with oil in lessening liver alterations in *Toxoplasma gondii* infected Swiss albino mice offspring.The findings indicate encouraging progress in maintaining liver tissue health and minimizing damage. Pregnant mice were divided into four groups (n=8 each): a control receiving distilled water, a group infected with *T. gondii* (5×10^5 tachyzoites via intraperitoneal injection on day 7 of pregnancy), a group treated with nanoemulsion from day 7 to day 18, and an infected group treated with nanoemulsion from day 7 to day 8. Scanning electron microscopy (SEM) confirmed the nanoemulsion's activity and stability. Garlic oil demonstrated the highest spiramycin solubility (31.47 mg/mL), making it a suitable carrier for the nanoemulsion formulation. Histopathological examination of fetal liver tissue revealed that untreated infected mice showed significant necrosis and vacuolar degeneration in liver cells. the group treated with spiramycin nanoemulsion exhibited marked improvement, with liver tissues appearing nearly normal and minimal inflammatory cell infiltration. The group treated with nanoemulsion without infection displayed liver histology comparable to the control group. This study concludes that spiramycin nanoemulsion, especially using garlic oil, effectively combats *T. gondii* and mitigates liver damage in fetal mice. The oral administration of the nanoemulsion offers a practical, non-invasive approach, providing a promising therapeutic strategy for managing congenital toxoplasmosis. These findings emphasize the potential of spiramycin nanoemulsion as a novel intervention for reducing the histopathological impact of *T. gondii* during pregnancy.

Keywords: Garlic oil, Istopathology, Swiss albino mice, Nanoemulsions, Spiramycin, *Toxoplasma gondi*

INTRODUCTION

The use of nanotechnology in treating some pathogens has an important and effective role, as it facilitates the delivery of the drug to its specific location and with minimal toxic effects or side effects, in addition to not affecting tissues close to the infection. Nanostructures also overcome resistance arising from the use of antibiotics, and the aforementioned technology also reduces of the doses required for treatment (Dos *et al*., 2018; Yeh *et al*., 2020).

T. gondii is common in many parts of the world and has veterinary and medical implications due to causes of miscarriages or congenital disorders in its intermediate hosts (Nayeri *et al.,* 2021). As a zoonotic pathogen, it has received substantial research. Tissue coccidia that create *T. gondii* cysts have a facultatively heterotrophic life cycle and can infect a variety of animals, including mammals, birds, and humans (Magana-Arachchi and Wanigatunge, 2020).

Asexual stages of the Toxoplasma-like parasite were first discovered in bird and mammalian tissues, and the first detailed description of *T. gondii* merozoites in rat spleen, liver, and blood was made in Africa (Pols, 2017). Cat feces may contain an infective stage of Toxoplasma gondii, which can induce infection when consumed by intermediate hosts (Hill and Dubey, 2018). Pathological alterations were observed in mice infected

with *Toxoplasma gondii* in brains at various times (Castaño Barrios *et al.,* 2021). They developed generalized meningoencephalitis on the second day, which persisted with varied degrees of severity. Lymphocytes and monocytes/macrophages comprised the inflammatory cells, whereas plasma cells dominated during the chronic phase (Suhrbier, 2019).

The brains revealed tiny foci of atrophic calcification. Toxoplasmosis is a major health concern in humans and animals that results from the parasite's obligate intracellular invasion of reticuloendothelial tissue (Daher *et al.,* 2021). Toxoplasmosis can affect many organs, including the host's liver, spleen, and neurological tissues. Although the parasite moves quickly to the liver during an acute infection, no substantial clinical changes can be seen (Masia and Misdraji, 2018).

Depending on the strain's virulence, the liver can undergo a variety of pathological alterations, including hepatomegaly, hepatitis, cholestatic jaundice, and fibrosis (Smith *et al.,* 2021). Toxoplasmosis treatment is required for pregnant women, people with acquired immunodeficiency syndrome, and those receiving chemotherapy, as parasites cause severe morbidity and mortality in these groups (El-Naggar, 2021). *Streptomyces ambofaciens* bacterium produces spiramycin. It is considered an antibiotic for parasites of the macrolide class (Smith *et al.,* 2021). It is used to treat Toxoplasma infection in pregnant women, and the outcomes are favourable (El-Naggar, 2021).

Nanomedicine is concerned with using nanotechnology to prevent, treat, and control diseases. Nanotechnology -based drug delivery methods are being used to improve drug kinetics (Peyron *et al.,* 2019). Enhancing solubility improves the drug's stability and solubility, as does its surface, and the treatment can be adjusted through membrane absorption, resulting in increased bioavailability at lower doses (Sahu *et al.,* 2021). The use of nanoemulsion drug delivery systems improves the bioavailability of hydrophobic medicines. Nanoemulsions and nano-oil dispersions offer several advantages, including improved absorption, penetration, and smaller dosages (Zhao *et al.,* 2019; Alabsy & Alabdaly, 2022). The study sought to explore the efficacy of spiramycin nanoemulsion in addressing the adverse histopathological effects of *T. gondii* on fetal livers in mice fetuses.

MATERIALS AND METHODS

Ethical approval

The Animal ethical approval was received from the Scientific Research Committee, College of Veterinary Medicine, University of Mosul (UM.VET.2023.081).

Animals

The study included 32 pregnant female mice (Swiss albino) weighing 28 ± 4 grams at 55 ± 8 days of age. The animals were obtained from Tikrit University, Tikrit. The presence of a vaginal plug was first established in all pregnant mice kept with males. The pregnant females were separated into special cages with plenty of animal feed and water, and the temperature in the laboratory was changed to maintain 12/12 hours of light and darkness.

Laboratory infection

Mice were injected intraperitoneally with a concentration of tachyzoites from *T. gondii* at 5 × 10^5 parasites per ml. Spiramycin was sourced from a Swiss business (BIOSYNTH).

Chemicals: Tween 80 and 20 from Riedel-De-Haen were acquired from a German manufacturer, while the dialysis membrane (12000 Da) came from Schcuhardt. All compounds, including solvents, were of analytical reagent grade.

Solubility and Saturation

Using several oils, including garlic, olive, corn, and sesame oil. Use surfactants (Tween 20 and 80), ethanol and methanol. Spiramycin was mixed with 5 ml of each oil and surfactant, agitated, and placed in a water bath for 72 hours at 25 ± 1 °C (Patel *et al*., 2024). When the equilibrium stage was reached, the solution was centrifuged at 2500 rpm for 15 minutes, filtered and diluted with ethanol, and the absorbance was measured using an ultraviolet meter.

Spiramycin nanoemulsion

Spiramycin nanoemulsion (NE) concentrations were created using Smix and oil, and spiramycin was dissolved by diluting 2 mg of the medication in the selected oil. Slowly and steadily add the prepared mixture until you get a clear solution. A light source was employed to test the transparency (Yadav *et al.,* 2018).

Scanning electron microscope (SEM)

A drop of Nanoemulsion Gel (NG) was applied to a copper-coated grid, allowed to dry, and then stained for 10 seconds. The surplus staining solution was subsequently removed from the grid with a Philips 208S transmission electron microscope (Saleh, 2019).

Fourier transform infrared analysis

Potassium bromide was used to fill a disk with pure medication, and the wavelength (400 to 4000 cm-1) was calculated. The spectrum data that required to be evaluated was collected, together with any discrepancies across samples (Vyas *et al*., 2009).

Validation of the High-Performance Liquid Chromatography (HLPC)method

HPLC was used to determine interactions between the

oil, drug, and other excipients. A water HPLC system equipped with a SPA-20A detector. Use Breez software was used.

Experimental design

The pregnant female mice were divided into four groups of eight each. The group (I) was designated as a control group and was given only distilled water. The group (II) received an intraperitoneal injection of 5 × 105/ml tachyzoites on the seventh day of pregnancy. The group (III) received an oral dose of spiramycin nanoemulsion 0.1 ml from the seventh to the 18th day of pregnancy. The group (IV) was injected with *Toxoplasma gondii* diluent intrapersonal and then treated with spiramycin nanoemulsion from the seventh to the eighteenth day of pregnancy.

Histological preparation

The embryos were obtained and then dissected to take their livers in day 19 of pregnancy. They were immersed in 10% formalin and then placed in 70, 80, 90 and 100% alcohol. All stages of histological sections were completed, and slides were prepared and stained with Hematoxylin and Eosin. An optical microscope was used to examine the samples.

RESULTS

Table 1 shows that among the oils, garlic oil exhibited the highest solubility of spiramycin (31.47 mg/ml), followed by olive oil (16.59 mg/ml), corn oil (11.94 mg/ml), and sesame oil (7.15 mg/ml). Surfactants demonstrated significantly higher solubility compared to oils. Tween 80 showed the highest solubility (85.31 mg/ml), followed by Tween 20 (42.54 mg/ml).

Co-surfactants also enhanced solubility, with ethanol showing a higher solubility (47.92 mg/ml) than methanol (26.08 mg/ml). The effectiveness of surfactants and co-surfactants was evident: surfactants like Tween 80 and Tween 20 significantly increased the solubility of spiramycin compared to oils. Tween 80 exhibited higher solubility compared to Tween 20. Co-surfactants, such as ethanol and methanol, also enhanced solubility, with ethanol showing higher solubility than methanol.

Choice of formulation components

For formulations that required high solubility of spiramycin, Tween 80 could have been preferred over Tween 20. Ethanol was identified as a suitable co-surfactant choice for spiramycin formulations requiring higher solubility.

Practical implications

The choice of solvent components (oils, surfactants, and co-surfactants) significantly influenced the solubility of spiramycin, which was crucial for formulation development. These findings could have guided the formulation process of spiramycin-based products, ensuring optimal solubility and potentially enhancing their efficacy.

Table 1. Spiramycin Solubility (mg/ml) in Different Oils, Surfactants, and Co-surfactants

Compounds		Solubility mg/dl
Oil	Corn	11.94
	Olive	16.59
	Garlic	31.47
	Seassme	7.15
Surfactant	Tween 20	42.54
	Tween 80	85.31
Co-Surfactant	Ethanol	47.92
	Methanol	26.08

Morphological notes

Samples observed under SEM showed that NE did not contain ceramides, consisting instead of dispersed particles of approximately 182 nm. (Fig. 1).

FTIR spectra

The typical peaks of FTIR spectra for pure spiramycin powder were observed at 3489.23 cm^{-1} due to vibrational stretching (N-H) and at 2924.09 - 2862.36 cm^{-1} related to stretching (=CH-H). Ester expansion (C=O) was responsible for the 1957.75, 1735.93, and 1643.35 cm^{-1} peaks.

Confirmation of the validity of the HLPC method

No color difference was recorded between spiramycin and spiramycin nanoemulsion. While no additional peaks were observed. This means that some of the excipients, such as Tween 80 and Tween 20 oils, ethanol, and methanol, are compatible with spiramycin. The spiramycin and spiramycin nanoemulsion chromatograms are shown in Fig. 2 and 3.

The chromatogram results show that the nanoemulsion contains a high concentration of spiramycin with a low concentration of impurities. Impurities refer to substances that are not part of the main compound (in this case, spiramycin) and may result from manufacturing processes, excipients, or any unwanted reactions) (Fig. 4, 5).

Histopathological examination

Fig. 5 displays the liver of fetuses from the control group, illustrating a typical central vein (CV) and sinusoids (S). Notably, Megakaryocytes (MKC) and hematopoietic elements (HPE) are observed, as highlighted by H&E staining at 400x magnification.

In Fig. 5, the liver of fetuses from the infected group is depicted, showcasing features of Vacuolar degenera-

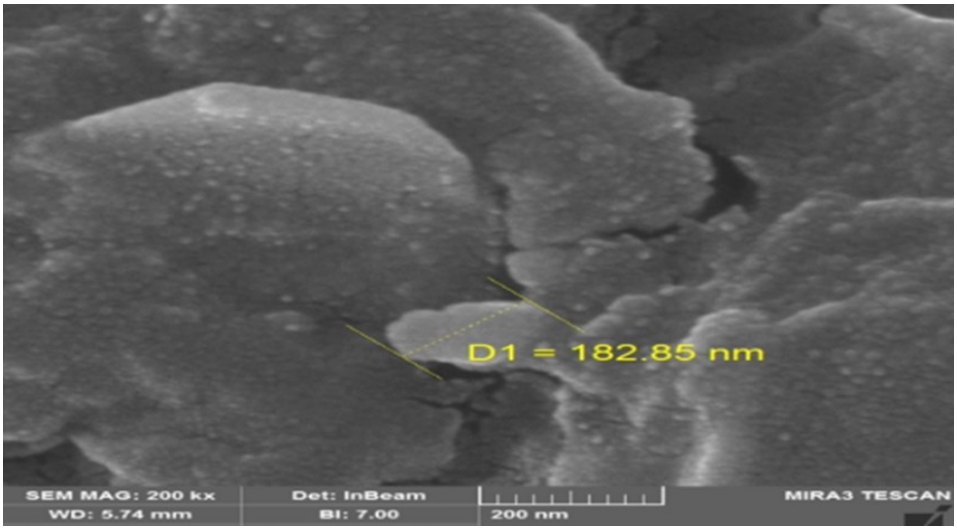

Fig. 1. *Scanning electron micrographs of spiramycin nanoemulsions used for drug delivery applications, showing particle size distribution and structural morphology*

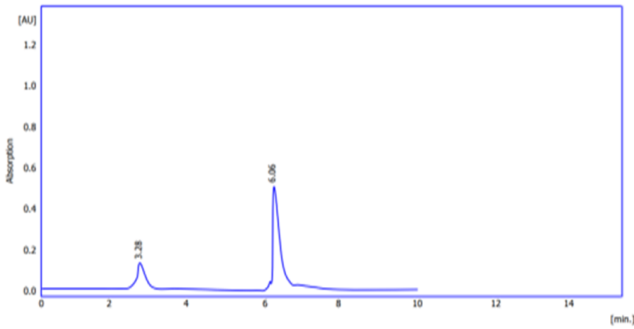

Fig. 2. *Chromatograms of spiramycin nanoemulsion*

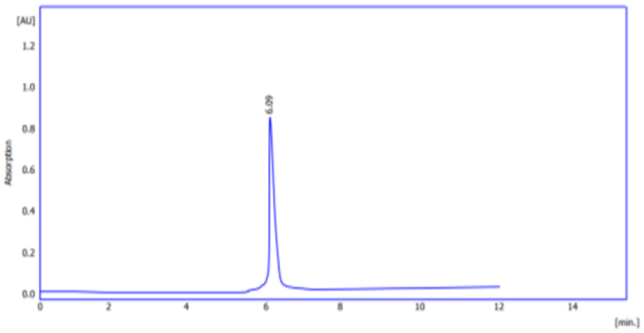

Fig. 3. *Chromatograms of spiramycin*

tion and necrosis of the hepatocytes

The liver of fetuses from the Spiramycin-NE group is presented in Fig.6, exhibiting characteristics akin to the control group, including the central vein (CV), hepatocytes (HC), and sinusoids (S). Furthermore, Megakaryocytes (MKC) and hematopoietic elements (HPE) are discernible within the sinusoids, as shown by H&E staining at 400x magnification.

Finally, in Fig. 7, the liver of fetuses from the treated group is depicted, showcasing similar features to those observed in the Spiramycin-NE group. Specifically, the central vein (CV), sinusoids (S), Megakaryocytes (MKC), and hematopoietic elements (HPE) are evident within the sinusoids, as illustrated by H&E staining at 400x magnification.

DISCUSSION

Drug dissolving rates are one factor that influences spiramycin's bioavailability. However, spiramycin's oral bioavailability is inconsistent and incomplete due to its low solubility and slow water dissolution rate in rats or mice model (Channabasavaraj *et al.,*2010; Abdul *et al*., 2023; Ahmed and Saleh, 2021). As a drug delivery device, Nanoemulsion is a potential technology for increasing the oral bioavailability of poorly soluble medicines and reproducing drug plasma concentration patterns (Alabbasi and Alabdaly, 2022).. As a result, it can be engineered to distribute medications in a small volume, provide a large surface area, and facilitate dissolution and penetration (Zhang *et al*., 2017).

In Fig. 5, the liver of fetuses from the infected group by *T. gondii* is depicted, showcasing features of Vacuolar degeneration and necrosis of the hepatocytes. The findings suggested that histological abnormalities in liver tissue could be caused by *T. gondii* interfering with mitochondrial activity and shifting to anaerobic techniques to provide enough energy for the operation of sodium pumps, decreasing protein output.

T. gondii can cause damage to the membranes of liver cells (hepatocytes). This disruption can lead to a loss of cell integrity and function, making it difficult for the cells to maintain their normal activities (Sutradhar and Amin, 2013; Al-Jammas *et al.,* 2024).

Phagocytosis is the process by which cells, such as macrophages in the liver, engulf and digest foreign particles, pathogens, or cellular debris. T. gondii can interfere with this process, reducing the ability of immune cells to clear infections or damaged cells effectively (Al-Sabawi,*et al*., 2018; Saeed *et al*., 2023).

As a consequence of the above effects, the liver tissue may experience necrosis, which is the death of cells in

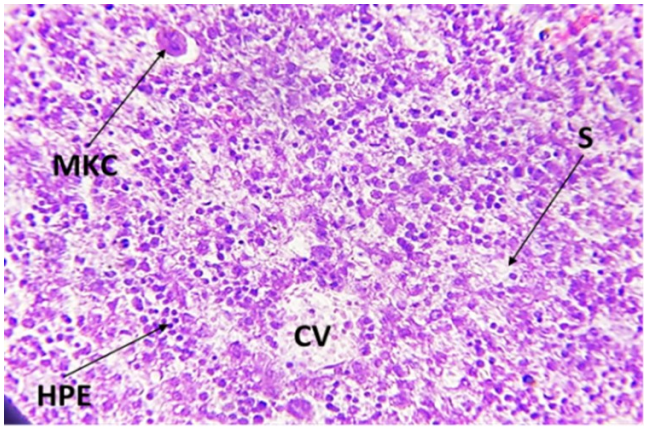

Fig. 4. *Liver of control group of fetus showing central vein (CV) and sinusoids (S) with presence of megakaryocytes (MKC) and hematopoietic elements (HPE) H&E stain, 400X*

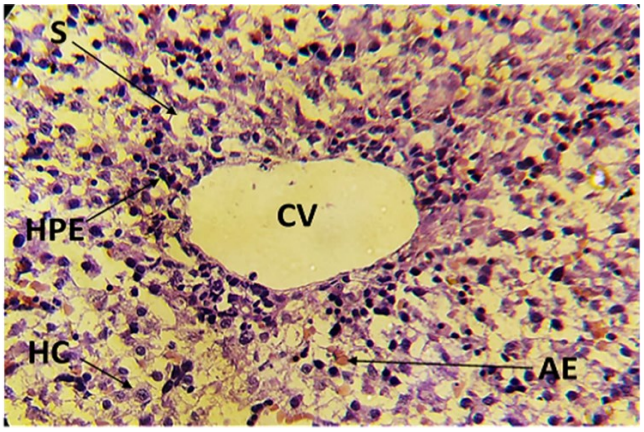

Fig. 5. *Fetus liver of infected group shows in the central vein (CV), hepatocyte (HC) sinusoids (S) with hematopoietic elements (HPE) and a nuclear erythrocytes (AE). H&E stain, 400X*

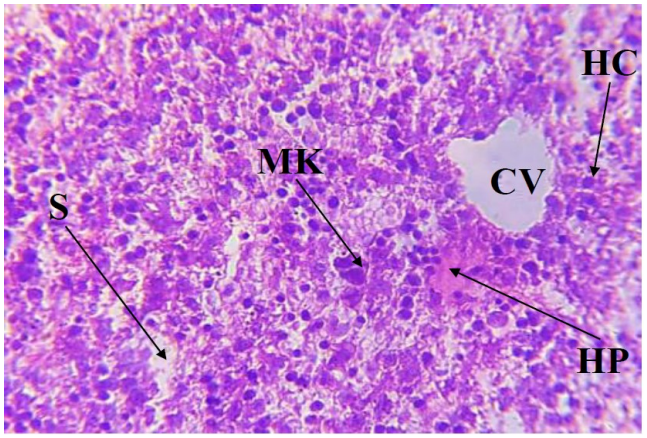

Fig. 6. *Fetus liver of Spiramycin-NE group shows the central vein (CV), hepatocytes (HC), sinusoids (S) with megakaryocytes (MKC) and hematopoietic elements (HPE) H&E X400*

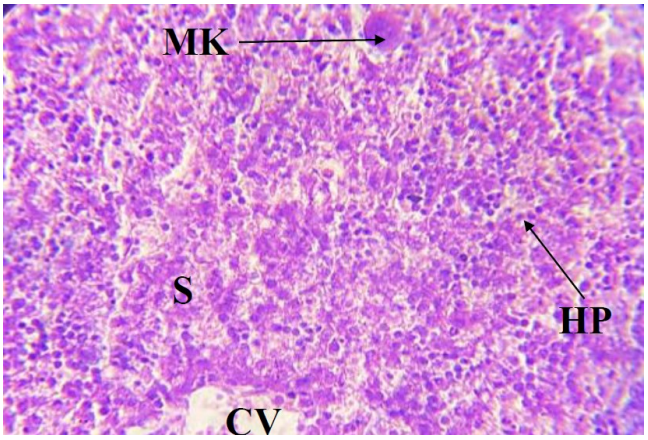

Fig. 7. *Fetus liver of treated group shows the central vein (CV), sinusoids (S) with megakaryocytes (MKC) and hematopoietic elements (H&E) H&E X400*

a particular area. Necrosis can result in inflammation and further damage to the liver, potentially impairing its overall function (Sutradhar and Amin, 2013; Ibrahim *et al*., 2024).

Findings imply that spiramycin nanoemulsion may be advantageous for boosting hematopoietic components. Many possible nanoemulsified medications have been described in the literature to deliver various pharmaceuticals, including anticancer, antiparasitic, antipsychotic, antiglaucoma, and statins (Laxmi *et al.,* 2015). Several researchers have earlier explored the objects of the nanostructures like nanoemulsions, solid lipid nanoparticles, polymeric nanoparticles, metal nanoparticles, and nanosuspensions on *T. gondii* at various phases *in vivo* and *in vitro* (Morsi *et al.,* 2017; Jafarpour *et al*., 2021).

The present study is distinguished by its focus on using nanoemulsions to deliver spiramycin against *T. gondii*, allowing for a deeper analysis of the effectiveness of this formulation. It also contributes to improving drug delivery and enhancing solubility and bioavailability compared to other methods while providing new insights into the mechanisms by which nanoemulsions enhance the action of spiramycin. The study also compares nanoemulsions and other nanocarriers comprehensively, offering broader applications for treating *T. gondii* infections.

The synergistic effects of specific nanoparticles and spiramycin on immunological responses to *T. gondii* demonstrated that the combined treatment was more effective than the individual treatment, the optimum consequence was found in mice that received a combination of chitosan and spiramycin-loaded silver nanoparticles (Hamad *et al.,* 2020). This supports the findings of the present investigation regarding the efficacy of spiramycin nanoemulsion against *T. gondii*.

The results of the present study support the findings of Hamad *et al.* (2020), showing that spiramycin effectiveness is improved when combined with nanoparticles to combat *T.gondii* infection. Though similar in some aspects to research, the uniqueness of the present work lies in utilizing spiramycin nanoemulsion optimized with

oil. A novel approach that has not been explored before.In addition, a new contribution comes from examining liver tissue histology in cases of toxoplasmosis, an aspect not addressed by Hamad *et al.* (2020). The present study also highlights the healing possibilities of using nanoemulsion orally while pregnant as a possibly more convenient method for treatment. These characteristics set the present research apart. Help progress the knowledge and usage of nanoparticle-centered treatments for toxoplasmosis.

Conclusion

The present research showed that using spiramycin nanoemulsion enhanced with oil effectively fought against *Toxoplasmosis gondii* and enhanced the health of livers in cases of congenital toxoplasmosis. Histological examination indicated a notable improvement in liver tissue health, with decreased necrosis and inflammation similar to those of the control group. The nanoemulsion administered orally during pregnancy demonstrated a practical and non-invasive treatment method, highlighted for its potential as an innovative solution for managing toxoplasmosis during pregnancy.

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Conflict of interest

The authors declare that they have no conflict of interest.

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