J Nanostruct 13(1): 29-36, Winter 2023

RESEARCH PAPER

Analyzing the Teratogenic Potential of Aluminum Oxide Nanoparticles (Al_2O_3NPs) On Albino Mice Fetuses

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ARTICLE INFO

Article History:
Received 11 October 2022
Accepted 25 December 2022
Published 01 January 2023

Keywords:
Al_2O_3NPs
Albino Mice Fetuses
Pregnancy
Teratogenic Effects

ABSTRACT

One kind of frequently utilized nanomaterials is aluminum oxide nanoparticles (Al_2O_3NPs). However, nothing is known about the teratogenicity and toxicity of Al_2O_3NPs in mammals. The goal of the current investigation was to determine whether this nanoparticle had teratogenic effects in albino mice during various stages of pregnancy. During this experimental study, 48 female albino mice were selected. Four experimental groups and two control groups were created by random selection among the subjects. Pregnant mice received an oral gavage dose of 8 mg/kg of Al_2O_3 solution from the first week through the 18th day of pregnancy. Al_2O_3NPs were administered orally to the first, second, and third groups solely during the first, second, and third weeks, respectively, and to the fourth group throughout the entire pregnancy. The fourth group received gavage mixed with a solvent in contrast to the control group, which received municipal water without gavage. The fetuses were removed from the fallopian tubes on the 18th day of pregnancy, and the morphological abnormalities, weight, height and head circumference of the fetuses were recorded. One-way ANOVA, Kruskal-Wallis test, and chi-square statistic were used for data analysis in SPSS version 23.0. A p-value less than 0.05 was considered statistically significant. Observations showed that teratogenic effects were more prominent in the group that was exposed to Al_2O_3NPs in the second week. The findings of this study demonstrate that Al_2O_3NPs have teratogenic and harmful effects in mammals.

How to cite this article
Roza A., Sadoon N., Kareem H L., Dawood F A., Mashool N M., Karim B R., Alabdallah Z A. Analyzing the Teratogenic Potential of Aluminum Oxide Nanoparticles (Al_2O_3NPs) On Albino Mice Fetuses. J Nanostruct, 2023; 13(1):29-36. DOI: 10.22052/JNS.2023.01.004

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INTRODUCTION

Nanoparticles (NPs) are very small particles that measure less than 100 nanometers in size. They have unique properties that make them useful in a variety of medical applications [1–3]. One of the main uses of NPs in medicine is in drug delivery [4]. Due to their small size, NPs can be designed to target specific cells or tissues in the body, allowing drugs to be delivered more effectively and with fewer side effects. For example, NPs can be coated with specific antibodies or peptides that allow them to bind to cancer cells, or to cells in the blood vessels of tumors, allowing drugs to be delivered directly to the site of the disease [5–7]. Another use of NPs in medicine is in imaging and diagnostic applications [8]. For example, gold NPs can be used to create contrast agents for imaging techniques such as computed tomography (CT) and magnetic resonance imaging (MRI) [9,10]. They also used in imaging techniques such as photoacoustic imaging, and fluorescence imaging, to detect the presence of disease [11]. Furthermore, NPs have been used in tissue engineering, regenerative medicine, and wound healing [12]. For example, NPs made of biodegradable polymers have been used to create scaffolds for growing new tissue, and as a delivery vehicle for growth factors and other molecules that promote tissue repair. It's worth noting that, while the use of NPs in medicine has the potential to offer significant benefits, more research is needed to fully understand the safety and efficacy of these materials in humans, and to establish safe exposure levels for patients and healthcare workers.

Aluminum oxide nanoparticles (Al_2O_3NPs) are spherical shaped NPs with a diameter < 10 nm [13]. They are used in a variety of applications due to their unique properties. One of the main uses of Al_2O_3NPs is in coatings and surface treatments. They can be used to create coatings that are highly resistant to wear and corrosion, making them useful for protecting metal surfaces in a variety of industrial settings [14]. Another important use of Al_2O_3NPs is in catalysts. They have been found to be effective catalysts for a variety of chemical reactions and are used in the production of chemicals such as ethylene and propylene [15]. Additionally, they are used in biomedical applications such as drug delivery, tissue engineering, and imaging and diagnostic [16].

There is ongoing research on the potential teratogenicity and toxicity of Al_2O_3NPs in mammals [17]. Some studies have suggested that Al_2O_3NPs may be harmful to developing organisms, as they have been shown to cross the placenta and accumulate in the fetal brain in animal models [18–21]. However, the studies are not yet conclusive and more research is needed to fully understand the potential risks. In terms of toxicity, studies in animal models have found that Al_2O_3NPs can cause lung inflammation and lung fibrosis when inhaled, and that they can cause liver and kidney damage when ingested [22–24]. However, it is important to note that the toxicity of Al_2O_3NPs may vary depending on the size, shape, and surface properties of the particles, and that the safety of Al_2O_3NPs in humans has not been fully established [25]. It's worth noting that, more research is needed to understand the potential risks associated with exposure to Al_2O_3NPs, and to establish safe exposure levels for humans. It is important to handle and use Al_2O_3NPs in a safe and responsible manner, in accordance with established safety protocols.

Pregnant women are more susceptible to the toxic effects of NPs [26,27]. When pregnant mice/BALB were exposed to the identical Al_2O_3NPs exposure, it resulted in acute and long-lasting lung inflammation, but it had no effect on non-pregnant individuals [28]. If the embryo is harmed at this time, recovery will be challenging or impossible. It is estimated that 10% of birth abnormalities are caused by environmental causes, and over 50 substances have been identified as human teratogens [29–31]. Therefore, a great deal of research has been done on the teratogenic consequences of NPs, with Al_2O_3NPs making up a smaller portion of them.

The purpose of the current investigation was to determine whether Al_2O_3NPs, after being consumed by the female, has any teratogenic effects on albino mouse embryos.

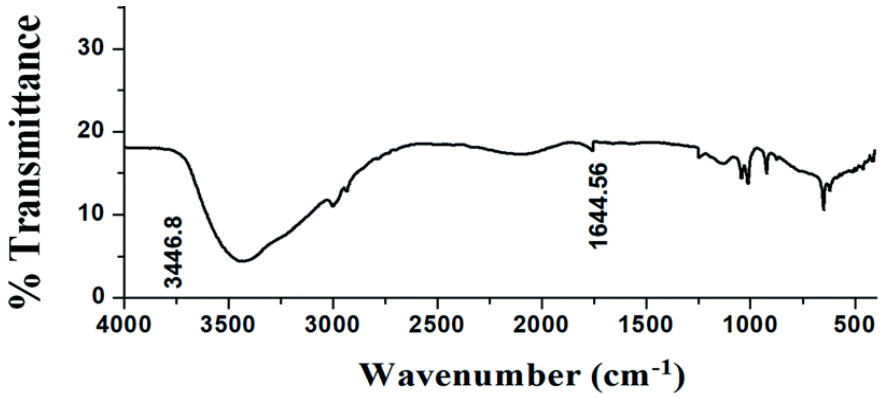
MATERIALS AND METHODS

This experimental study was carried out in 2021 on albino mice at the Cairo Infertility Center. The Al_2O_3NPs employed in this study had particle size of 16.7 nm range and was obtained from Alpha Chemika, Cairo, Egypt. The FTIR analysis in Figure 1(a) illustrates the peaks at 500-1000 cm^{-1}, which are characteristics of the (O-Al-O) bonds found in Al2O3 nanoparticles. Additionally, the distinct sharp peak at 1644.56 cm^{-1} is indicative of the (aromatic rings C=C) bonds present in the carbon

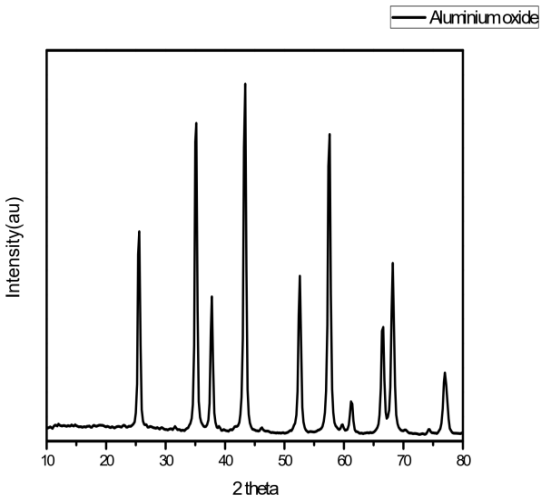
skeleton. X-Ray Diffraction (XRD) was used to check the characterization of the chosen NPs, and it was discovered that the particles are clean and devoid of contaminants (Fig. 1(b)). Transmission Electron Microscope (TEM) scans of Al_2O_3NPs showed that the size and shape of the particles were irregular, crystalline, and somewhat symmetrical (Fig. 1(c)).

48 mice (28±4 grams, 6-10 weeks of pregnancy) were used in this experimental study. Observing the emergence of a vaginal plaque that indicated gestational day 0 (GD0) served as confirmation of pregnancy. The pregnant females were housed separately in plastic cages with enough food and water, a temperature range of 23–27°C, a humidity range of 42–51%, and a 12-hour cycle of darkness and light. Six groups of eight mice each received a different treatment after being randomly assigned to groups: control (city water via gavage), sham (nano PBS phosphate buffer saline via gavage), and target groups (first, second, third and fourth groups). During pregnancy days between 8 and 10 a.m., the target groups received a gavage of an Al_2O_3 solution with an average dose of 8 mg/kg through a gastric tube. The first group will receive the gavage during the first week of pregnancy, the second group during the second week, the third group during the third week, and the fourth target group will receive the gavage throughout the entire pregnancy.

According to the ethical guidelines for working



(a)



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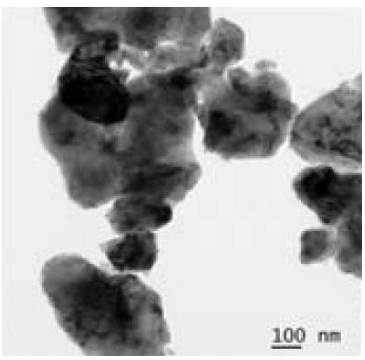

(c)

Fig. 1. Analysis results of Al_2O_3NPs: (a) FTIR spectroscopy, (b) XRD image, and (c) TEM image.

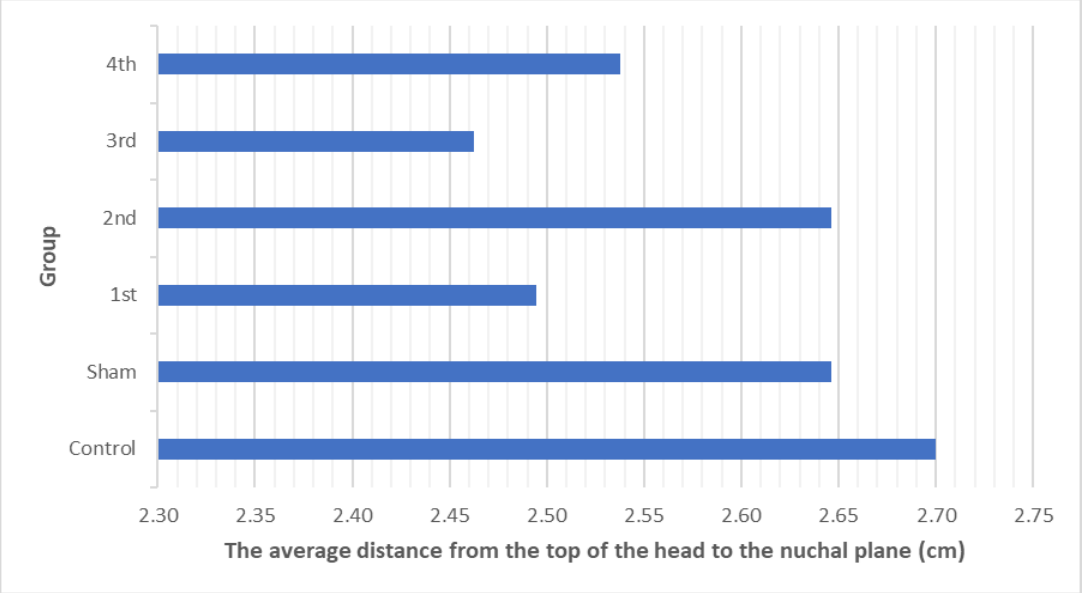

Fig. 2. Comparing average crown rump length (CRL) between groups.

with animals, the pregnant female mice were killed on the 18th day by dislocating their necks. The abdominal region was then dissected, and the embryos were taken out one at a time and weighed in a petri dish containing physiological serum on a digital scale. The distance from the top of the head to the nuchal plane, as well as the anterior and posterior aspects of the head circumference of the fetus, were measured and calculated using a caliper. The number of fetuses, fetal deaths, and structural malformations of the skeleton and limbs, polydactyly, cranial malformations, atrophies, subcutaneous bleeding, placental abnormalities, and other morphological abnormalities were recorded and measured. This section was completed using the OECD-GLP guidelines [32]. The Alizarin Red S double staining procedure (for bone) and Allison Blue (for cartilage) were used in histopathological tests to assess bone density, ossification patterns, ossification centers, delays in growth or congenital defects in organs and bones, and the normal development of bones and cartilages [33,34]. This technique involves coloring the cartilages blue with Allison Blue and the bones red with Alizarin Red S. The materials were stained, made transparent, and then inspected under a stereo microscope while being photographed with a stereo microscope equipped with a camera. The statistical software SPSS version 23.0 was used for data analysis. Chi-square test was used to compare and evaluate qualitative variables, while one-way ANOVA was used to compare and evaluate quantitative variables if the data were normal. The Kruskal-Wallis test was applied in the event of non-normality. A p value <0.05 was regarded as significant.

RESULTS AND DISCUSSION

In the macroscopic observations, factors such as fetal weight, the distance from the nuchal plane, head circumference, and changes in maternal weight before and after pregnancy were examined. The average weight, crown rump length (CRL) and head circumference of 18-day-old fetuses whose mothers received oral Al_2O_3NPs at a dose of 8 mg/kg, had a significant decrease compared to the control and sham groups that did not receive this dosage ($p<0.05$) (Fig. 2). Additionally, the Kruskal-Wallis test revealed that among the examined groups, the third week group had fewer fetuses on average than the other groups. In comparison to other test groups, the third week group's average head circumference was smaller.

Fertility rate in female albino mice that were treated with Al_2O_3NPs in the first mating, after the formation of vaginal plaque and confirmation of pregnancy, was reduced by 83%. Only one mouse in the treatment groups—which were

Table 1. Comparison of the frequency of dead and live embryos between groups.

Group	Live embryos, %	Dead embryos, %
Gavage entire period	78.07	21.93
Gavage 1st week	94.25	5.76
Gavage 2nd week	97.64	2.36
Gavage 3rd week	90.64	9.36
Gavage sham	100	0
Gavage control	100	0
Total	94.35	5.65

administered gavage starting on the day of GD0—continued its pregnancy out of the five mice with vaginal plaque during the first week and the entire period. The mortality rate and the proportion of dead and absorbed embryos were significantly different in each group, according to the chi-square test ($p<0.05$). With 21.93% deceased fetuses, the group that received Al_2O_3NPs through gavage during the entire period had the highest death rate (Table 1).

The chi-square test revealed that there were statistically significant differences in the frequency of bleeding and bleeding abnormalities, such as subcutaneous bleeding, internal bleeding, and amnion, between the various test groups and the control and sham groups ($p<0.05$) (Fig. 3).

The frequency of these abnormalities in the second week group with 19.73% cases was higher than other groups. The incidence of limb abnormalities and polydactyly, including improper twisting and shortening of the upper and lower limbs, was statistically different between the control and sham groups, according to the chi-square test ($p<0.05$). The frequency of incidence of these anomalies in the entire period group compared to the control and sham groups showed a significant difference ($p<0.05$), as shown in Fig. 4.

Fig. 5 shows a significant difference in

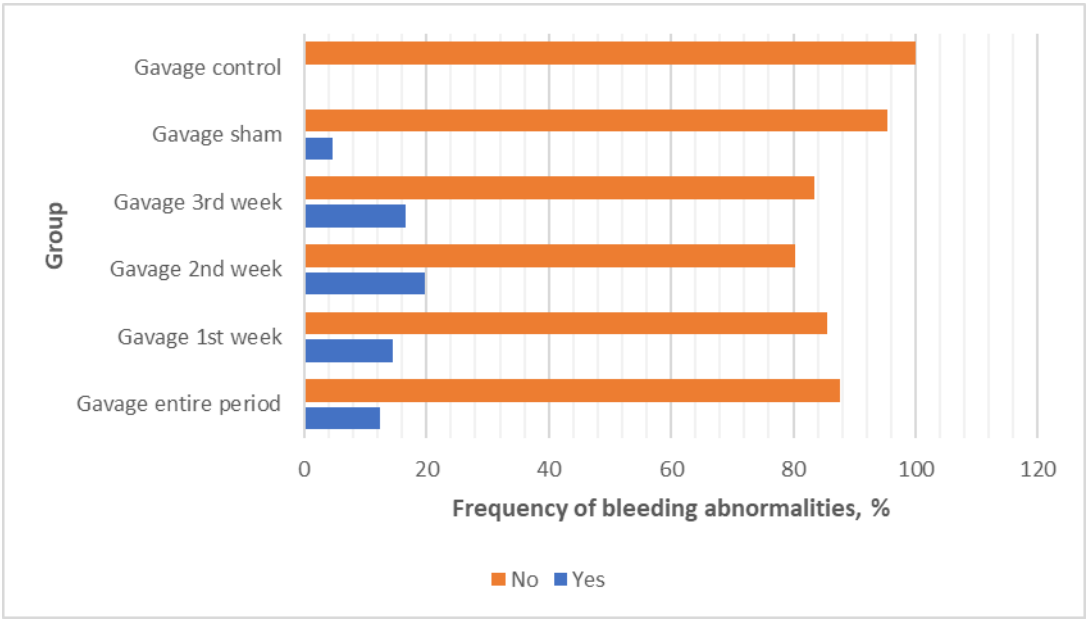

Fig. 3. Monitoring the occurrence of bleeding abnormalities.

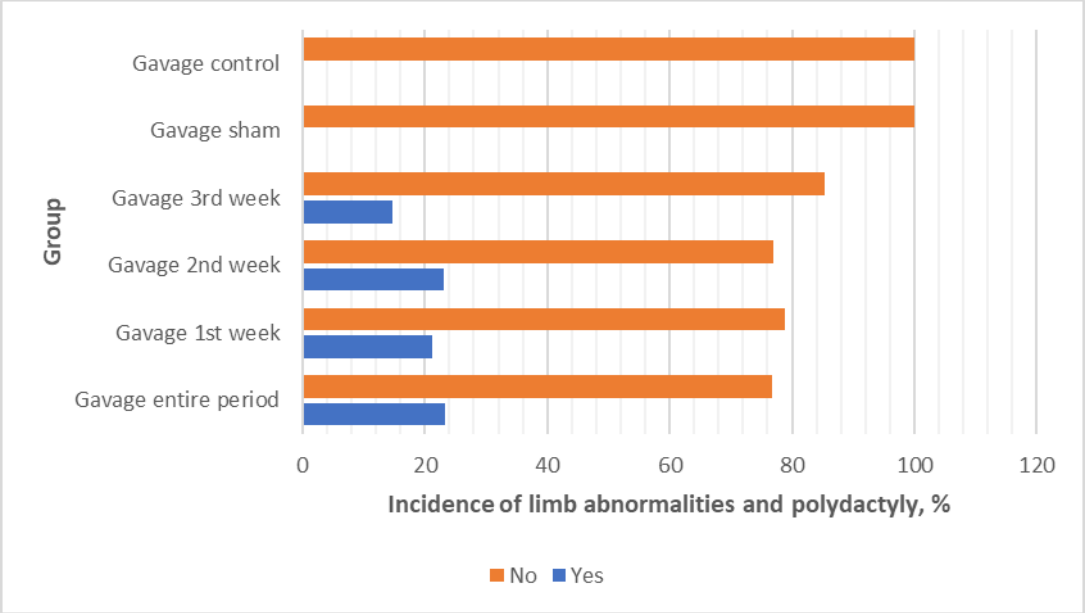

Fig. 4. The incidence of limb abnormalities and polydactyly.

the prevalence of skeletal and cartilaginous abnormalities between the test groups and the sham and control groups ($p<0.05$).

Nanomaterials differ from macro materials in terms of their physical and chemical characteristics due to their nanoscale size since the most significant change at this scale is the rise in the surface-to-volume ratio [35,36]. Today, pollution caused by nanoparticles has been raised as a new and dangerous problem [4]. Al_2O_3NPs can enter the human body through a variety of routes, including inhalation and skin contact [17].

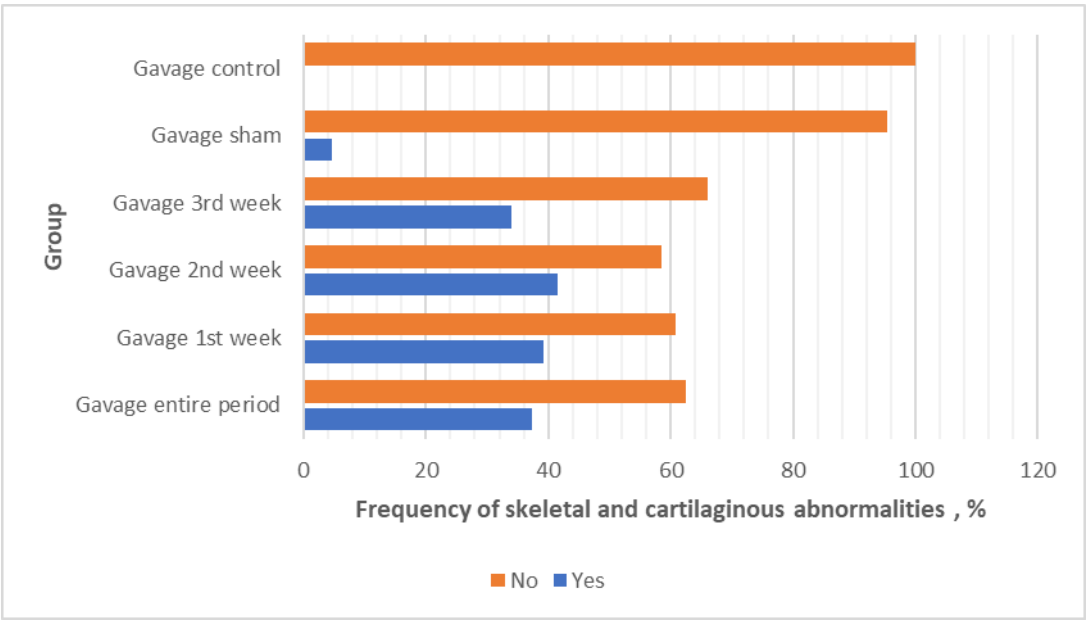

Fig. 5. The frequency of skeletal and cartilaginous abnormalities between groups.

To evaluate the fetal growth, various indicators are used, such as the length of the distance from the top of the head to the nuchal plane (CRL), weight and circumference of the head. In this study, the average of each of the three parameters was compared between various test and control groups. In the current study, a significant difference between the average body weight of the fetuses in the test groups and the control group was found. This difference clearly demonstrated a decrease in the weight, body length, and head circumference of the fetuses in the groups treated with Al_2O_3NPs ($p<0.05$). In the 3rd week, Al_2O_3NPs had the biggest impact on the weight of the fetuses in the treatment group. Al_2O_3NPs have numerous harmful effects on developing embryos and can cause tissue apoptosis and necrosis by blocking growth- and development-related enzymes as well as free radical production [13]. The placenta is affected by NPs, which also results in neutrophil activation, systemic inflammation, and malfunction, placental abortion, and fetal growth restriction [27].

CONCLUSION

There is ongoing research on the potential effects of Al_2O_3NPs on the fetus during pregnancy. Studies in animal models have shown that Al_2O_3NPs can cross the placenta and accumulate in the fetal brain. However, the studies are not yet conclusive and more research is needed to fully understand the potential risks. In this study, the skeletal and neurological systems of the developing fetus are significantly impacted by Al_2O_3NPs, which also have teratogenic effects on the fetus in various weeks. Additionally, at a dose of 8 mg/kg of the mother's body weight, this drug slows fetal growth and decreases fetal weight. The relationship between this nanoparticle's hemorrhagic problems and the developing fetus's blood system is clear. The fetal defects of this material are more obvious in the second week of mouse pregnancy due to its extremely small size and surface features.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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