Effect of Microencapsulated Hepatocytes Transplantation on Copper Metabolism of Rat Model with Hepatolenticular Degeneration

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ABSTRACT

Objective: To observe the effect of microencapsulated hepatocytes after intraperitoneal transplantation of hepatolenticular degeneration (HDL) of model rats' serum copper and copper metabolism in liver.

Methods: Rat HLD model was made by copper load diet. To prepare microencapsulated hepatocytes by the airflow method, and the method of intraperitoneal transplantation were used with naked cells or the microencapsulated cell transplantation, respectively, compared with blank control group group I), model group (group II), Bare hepatocytes after intraperitoneal transplantation group (group III) and microencapsulated hepatocyte transplantation group (group IV). Determination of HLD rat model of ALT, AST, albumin levels, copper of liver tissue, copper level in serum.

Results: II, III, IV groups of rats at each time point of ALT, AST, serum copper, liver copper values were significantly higher than group I rats increased (each $P < 0.05$), synthesis of albumin levels decreased significantly (each $P < 0.05$).

Keywords: Copper metabolism, hepatocyte, hepatolenticular degeneration

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West Indian Med J 2015 **DOI: 10: 7727/wimj.2015.433**

Conclusion: Microencapsulated hepatocytes intraperitoneal transplantation can significantly reduce the liver copper deposition of HLD rat, accelerate metabolism of serum copper, may become a new method for cell transplantation in the treatment of HLD. The ALT, AST, serum copper and liver copper levels of group IV were significantly lower than that in group III after 7-14d (each $P < 0.05$), the lowest value respectively with 85.1±7.0 U/L, 87.9±22.7 U/L, 2.44±0.18μg/ml, 26.73±3.22μg/g, and albumin in 14d than that in group III, the most high up to 38.36±1.52g/L

INTRODUCTION

Hepatolenticular degeneration (HLD) is a genetic disease with copper metabolic disorder characterized by multiple organ involvement, occur in adolescents, often early involvement of liver (1), which is relatively common in China (2). Hepatocyte transplantation has been becoming one of the treatment scheme of HLD (3, 4). Hepatocyte microencapsulation technology enables allogeneic cell transplantation becomes possible, which can effectively avoid immune rejection and prolong the lifespan of liver cells (5,6). In this artical, to study its effect on copper metabolism of HLD rats through the comparison of HLD microencapsulated rat hepatocytes after intraperitoneal transplantation and naked hepatocyte transplantation.

Materials and methods

Materials: Trypsin, Type I collagenase, EGTA, Sodium alginate, Poly lysine, Percoll(Sigma company, USA), DMEM culture medium (glucose, Hyclone company, USA), EDTA(Amresco company, USA), Trypan blue(Beijing Suolaibao company).

Isolation and culture of primary rat hepatocytes

Liver tissue from adult male Wistar rats (provided by the experimental animal center of Wenzhou Medical University, approved by the ethics committee of Wenzhou medical university), hepatocyte isolated by EDTA-collagenase in situ two steps perfusion method(7), The method is as follows: Rats were anesthetized, with D-Hank's solution for portacaval bypass perfusion, perfusion of about 30ml/min,

about 7 minutes, waiting for the liver was soft opened, vena cava cannula outflow liquid turbid, stop infusion. Remove the liver, in DMEM medium to tear the liver capsule, hepatic cells gently shake off into the medium, followed by repeated centrifugation washing 2 times, liquid into cell suspension cultured with DMEM, Percoll separation of liquid specific gravity 1.096 purified hepatocytes. Obtained cell contained by DMEM complete culture medium of 10% fetal bovine serum and 1% penicillin streptomycin, cell counted and determined the activity after dyeing 4% trypan blue, each rat hepatocyte yield can reach more than 10^8 cells, when the cell activity of >85% preparing for microencapsulation.

Cell Microencapsulation

The preparation of microcapsules use of one-step process microcapsule-forming method with sodium alginate barium chloride(8). Centrifugal collection of hepatocytes, and mixed 1.5% purified sodium alginate solution into the air-flow method, microcapsule generator homemade, gas flow rate of 4L/min, sodium alginate solution drop speed is 50ml/h, drip into the 25mmol/L $BaCl_2$ solution. Sodium alginate and Ba^{2+} cross-linked into capsules, and static 15min. Washed by D-Hanks liquid for 3 times to remove the excess $BaCl_2$, into DMEM culture medium for culturing.

Establishment and experimental grouping of rat HLD model

Rat HLD model was established by copper loading method(9). Took 120 male

Wistar rats of 3 months old, according to the requirements of the experiment were randomly divided into model group (II group), bare hepatocytes after intraperitoneal transplantation group (group III) and microencapsulated hepatocyte transplantation group (group IV), divided into 5 time points with 3 days, 7 days, 14 days, 21 days, 28 days (copper load for ninth weeks as starting 0 days), each group 8 rats for per time point, another 8 rats as the blank control group (group I). All rats were fed for 12 weeks according to the standard, group II, group III and group IV were fed with feed containing copper sulfate 1g/kg and 0.185% copper sulfate water also, a total of 12 weeks. From ninth weeks of feeding, took the rat median abdominal incision 5mm, group II, group III and group IV according to the experimental requirements were respectively injected 0.9% sodium chloride solution 2ml, bare hepatocytes and hepatic cell microcapsules, with 12 gauge needle. Each rat of group III and group IV was peritoneal injected with approximately 1×10^7 liver cells. After operation strict disinfection of wounds was made.

Index Determination

ALT, AST, albumin determination were taken at different time points: the serum samples of Wistar rats, the value of ALT, AST, albumin was determined by automatic biochemical analyzer.

Determination of the content of serum copper: serum copper to be detected to measure serum 1:10 diluted with deionized water mixing, with atomic absorption

spectrophotometry measurement. Liver tissue copper determination: first with 0.9% saline repeatedly washed out of the liver tissue, then clean and dry with filter paper and weighed 500mg, concentrated nitric acid (analytical) 10ml with low temperature heating digestion, and completely dissolved to yellow clear transparent, the determination of copper content by atomic absorption spectrometry.

The Statistical Method

Data processing and statistical analysis using SPSS 16 statistical software package, samples compared with single factor analysis of variance and Newman-Keuls test.

Results

Primary hepatocytes and cells in microencapsules

Isolated Primary hepatocytes were cultured for 2d when viability of >85% preparing for microencapsulation.（Figure 1，2）

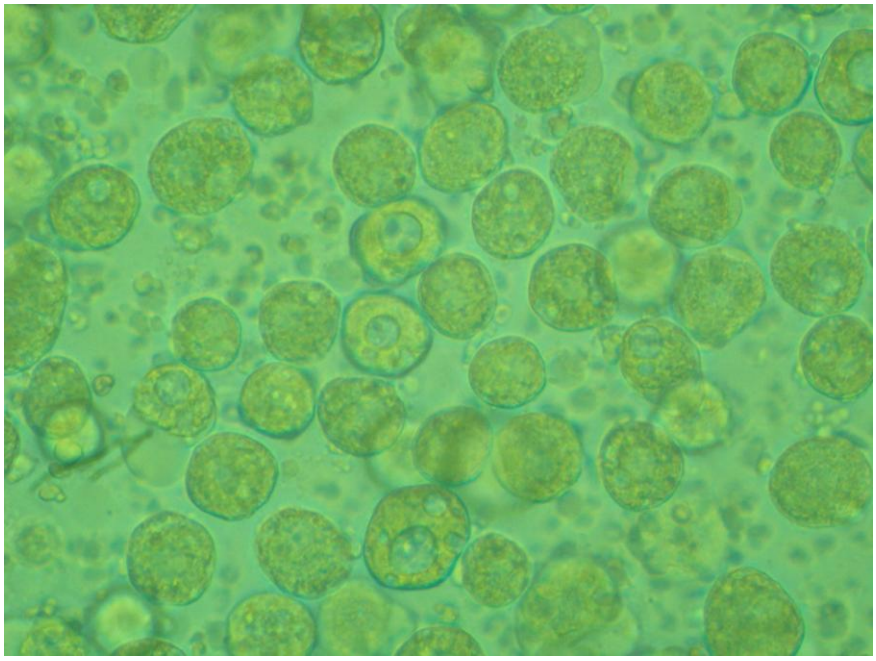

Figure 1- Isolated Primary hepatocytes
Hepatocytes yield >2.5×10^8/rat, when cell viability of >85% preparing for microencapsulation.

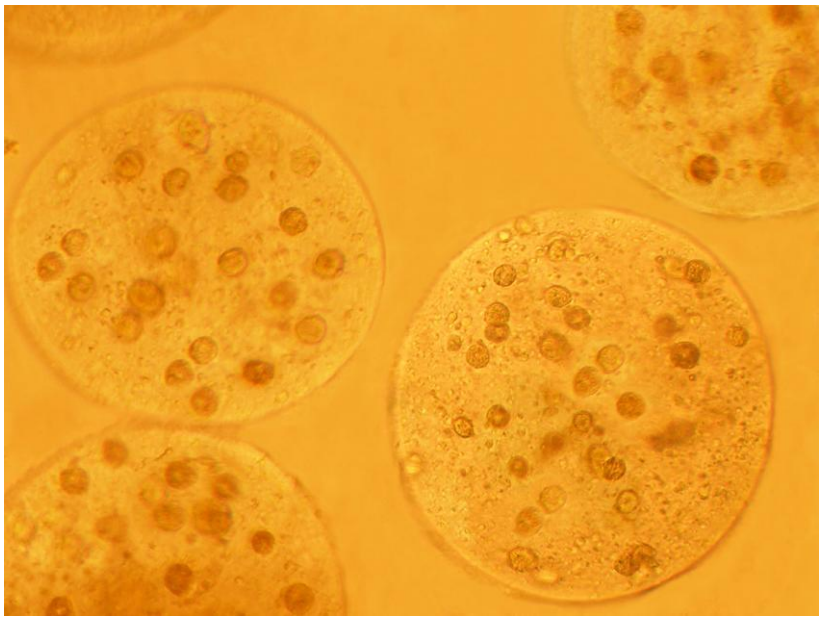

Figure 2- Cells in microencapsules
The microencapsule diameter of 0.4 ~ 0.8 mm include hepatocytes.

Serum ALT, AST and albumin variations

II, III, IV groups of rats at each time point of ALT, AST were higher than that in normal group rats (each $P<0.05$), synthesis of albumin levels decreased significantly

(each P<0.05). The ALT, AST level of group II rats increased gradually with time, and albumin levels declined gradually. There was significant difference between group III and group II rats of ALT, AST, albumin levels at the corresponding time point for compared (each P<0.05). The rats in group III ALT when 7d is at its lowest point in 117.3 + 9.6 U/L (compared with the same group of other time point P<0.05), and albumin levels in the 7d reached the peak of 33.96 + 1.73g/L (compared with the same group of other time point P<0.05). The rats of group IV in 3d compared with group III, ALT showed no significant difference (P>0.05), AST levels were higher than those of group III (P<0.05), albumin levels were lower than group III (P<0.05). When 7d group IV rats of ALT , AST level was significantly lower than that in group III (P<0.05), the lowest in 21d, were 85.1 + 7 U/L, 87.9 + 22.7 U/L, and albumin levels increased obviously, reached the highest to 38.36 + 1.52g/L in 21d also, but compared with group I, which still significant differences (P<0.05). (Figure 3, 4, 5)

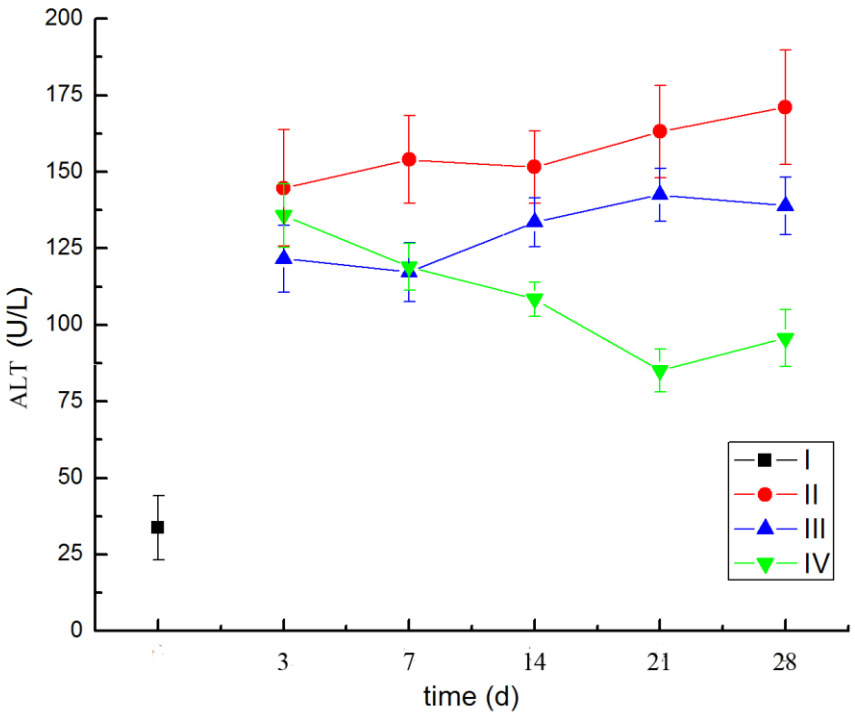

Figure 3- ALT value of each group at different time points

II, III, IV groups of rats at each time point of ALT was higher than that in normal group rats (each $P<0.05$). There was significant difference between group III and group II of ALT levels at the corresponding time point (each $P<0.05$). Group IV in 3d compared with group III, ALT showed no significant difference ($P>0.05$), When 7d group IV of ALT level was significantly lower than that in group III ($P<0.05$).

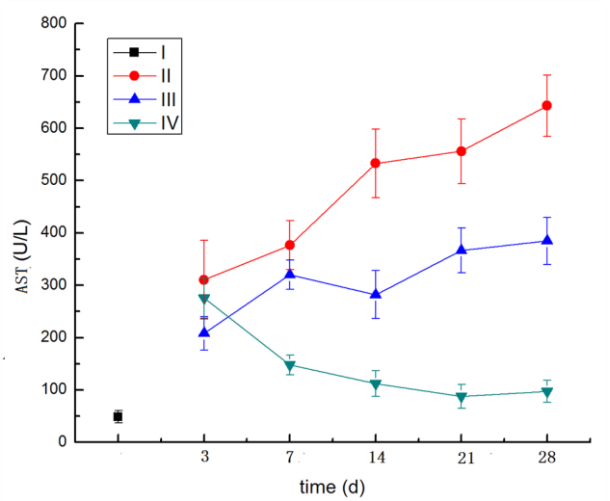

Figure 4- AST value of each group at different time points

II, III, IV groups of rats at each time point of AST was higher than that in normal group rats (each $P<0.05$). Significant difference between group III and group II of AST levels at the corresponding time point for compared (each $P<0.05$). The rats of group IV AST levels were higher than those time point of group III ($P<0.05$).

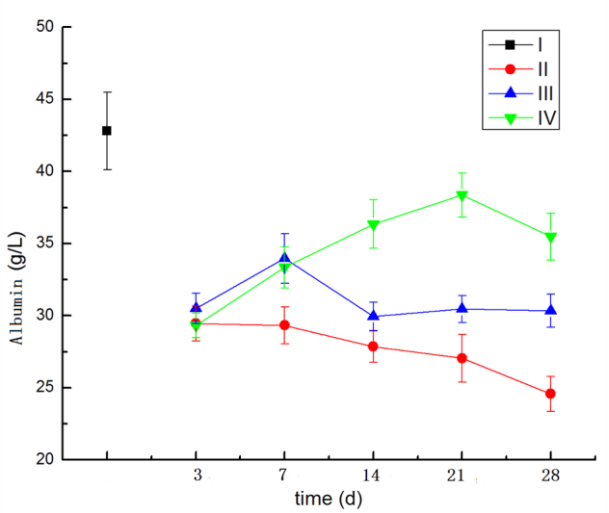

Figure 5- Albumin value of each group at different time points

II, III, IV groups of rats at each time point of albumin levels decreased significantly (each $P<0.05$). There was significant difference between group III and group II of albumin level (each $P<0.05$). Group IV in 3d compared with group III, albumin levels were lower ($P<0.05$).

Variations of serum copper, copper content in the liver tissue

II, III, IV groups of rats' serum copper, liver copper at each time point levels were higher than group I which significantly increasing (each $P<0.05$). The rats in group II, the liver copper content and serum copper increased gradually with the time point. Serum copper value of group III compared with group II at the corresponding time points were decreased (each $P<0.05$), and the liver copper content in 7d, 14d and 28d lower than that of group II (each $P<0.05$). Serum copper value of group III didn't get significant fluctuations after 7d ($P>0.05$), and the liver copper values in the 7d reaches the lowest to 36.10 + 4.4μg/g and thereafter gradually upward trend. Serum copper in group IV in 3d higher than that in group III ($P<0.05$), but after 7d were all lower than group III (each $P<0.05$), the minimum to 2.44 + 0.18μg/ml, but still significantly higher than those in group I ($P<0.05$). Liver copper content of group IV in 3d, 7d compared with group III showed no difference (each $P>0.05$), but the 14d decreased ($P<0.05$), the minimum to 26.73 + 3.22μg/g, were significantly higher than that of group I ($P<0.05$) . (Figure 6,7)

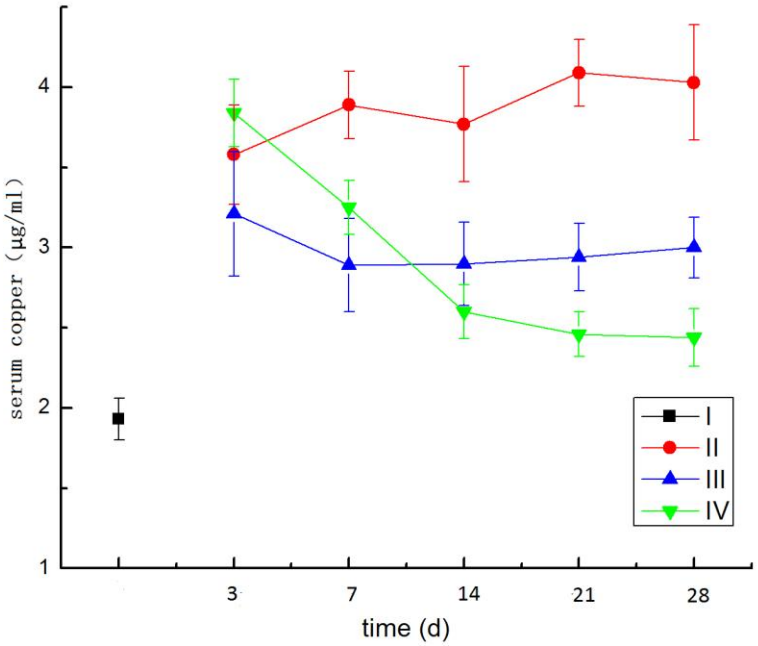

Figure 6- Serum copper value of each group at different time points

II, III, IV groups of serum copper at each time point levels were higher than group I (each $P<0.05$). Serum copper value of group III compared with group II were decreased (each $P<0.05$). Serum copper in group IV in 3d higher than that in group III ($P<0.05$), but after 7d were all lower than group III (each $P<0.05$).

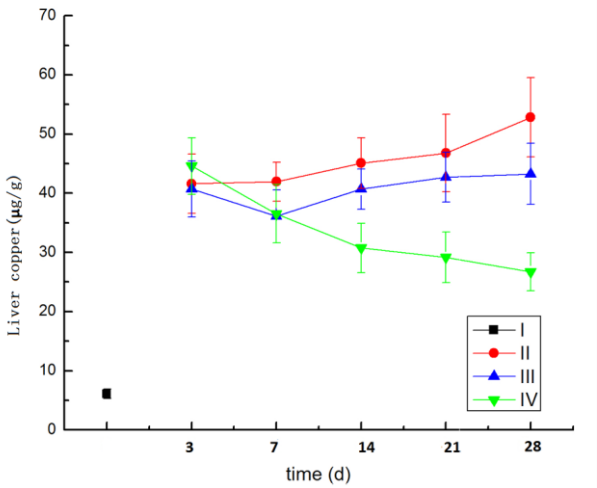

Figure 7- Liver copper value of each group at different time points

II, III, IV groups of liver copper at each time point levels were higher than group

I which significantly increasing (each $P<0.05$). Liver copper content of group III in 7d, 14d and 28d lower than that of group II (each $P<0.05$). Liver copper content of group IV in 3d, 7d compared with group III showed no difference (each $P>0.05$), but the 14d decreased ($P<0.05$).

Conclusion

Hepatolenticular degeneration (HLD) also known as Wilson disease, is a genetic disorder of copper metabolism caused by cirrhosis and in basal ganglia brain degeneration mainly autosomal recessive inherited disease(10,11). Children stage mainly shows liver damage, may display for the liver functional damage, cirrhosis, liver failure and so on, are the main diseases of childhood cirrhosis(12). Surgical treatment especially in liver transplantation, HLD becomes one of metabolic diseases can be cured(13,14). However, due to the difficulty of the operation and the limitation of donor liver source, living liver transplantation can not carry out extensive.

Hepatocyte transplantation is a kind of transitional replacement therapy of orthotopic liver transplantation (OLT) , because of wide cell source (such as pig), and transplantation path feasibility, also can reconstruct the copper metabolism balance in the body, reverse HLD process(15). But no matter what the liver and liver cells in vivo transplantation, immune rejection is the most important factors which still affecting the development of transplantation technology problem(16,17). In recent years developed microencapsulated hepatocytes technology with immune isolation barrier effectively, the research on cell transplantation has been paid more and more attention. Microencapsulated hepatocyte technology means hepatocyte is wrapped or isolated with selective semipermeable membrane(18), cells need to survive nutrients,

oxygen, metabolites and secretion of bioactive substances through a semipermeable membrane access, but host immune cells, immune globulin and complement can not through a semipermeable membrane, so hepatocyte in capsule will not suffer from host immune rejection and get long-term survival, exerts its biological function, to achieve the purpose of treatment. At present in the treatment of fulminant hepatic failure have got a high embodiment of value(19,20). The current HLD treatment has formed the hepatocyte transplantation in spleen transplantation method as the main pathway(21). But cause of the size which restricted to microencapsulated hepatocytes (300 microns), spleen transplantation is obviously difficult to achieve, therefore this research selected peritoneal transplant operation- the most common pathway for microencapsulated transplantation.

Copper load model is currently the most common HLD animal model, can better reflect the similar HLD liver copper injury(22). In this study, high concentrations of copper fed rats for up to 12 weeks in the given, the serum levels of ALT, AST and albumin levels showed a similar hepatitis even liver dysfunction performance, which is consistent with the liver damage after HLD copper deposition(23). Liver copper and serum copper level is the direct reflection of liver copper deposition to lead liver damage extent, meanwhile, monitoring of copper level in liver tissue and serum helps to understand the effect of hepatocyte transplantation therapy. In this experiment, the treatment group (naked hepatocyte transplantation group and microencapsulated hepatocyte transplantation group) liver aminopherase levels in rats after transplantation than copper loaded rats have decreased, liver copper and serum copper

levels also decreased, and the liver synthesis of albumin levels have also been enhanced. However, the naked hepatocyte transplantation group whether liver aminopherases, liver copper and serum copper level was decreased, or albumin synthesis recovery degree, or the effect of the consolidation time, compared microencapsulated hepatocyte transplantation group to the existence of significant differences. This may be due to immune bare hepatocytes can not tolerate receptor rejection. In this study, the decreasing degree of naked hepatocyte transplantation group liver aminopherase levels, copper levels were lowest in about 7 days, and 14 days is obviously aggravated, suggest that the function of the liver cells difficult to last more than 1 weeks. The microencapsulated hepatocyte transplantation rats liver aminopherases, serum copper, liver copper levels were significantly lower than the naked hepatocyte transplantation group, 28 days before took on a declining trend, while the synthesis of albumin levels increased significantly, at the same time prolonging holding time effect. This comprehensive experimental results showed microencapsulated hepatocyte transplantation can improve copper metabolism level of HLD rats, alleviate the hepatic copper deposition, accelerate the serum copper metabolism, improve liver function, indicates that the research prospect in hepatocyte transplantation in the treatment of HLD. But HLD is a chronic disease, how to further improve the time to maintain the function of hepatocyte transplantation will be the next focus of research work.

Acknowledgements

This work was supported by Medical science and technology project of Zhejiang Province, China (No. 201339321).

Conflict of Interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the position presented in, or the review of, the manuscript entitled,

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