

# Critique and recommendations for an experimental study

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Dear Editor,

I read with great interest a recently published article entitled “Effects of black cumin seed oil on oxidative stress and expression of membrane-cytoskeleton linker proteins, radixin, and moesin in streptozotocin-induced diabetic rat liver” in *Hepatology Forum*.<sup>[1]</sup> The authors presented the results of an experimental diabetic study by considering the distribution and expression level of membrane cytoskeleton linker proteins radixin and moesin. They also reported a possible protective effect of black cumin seed oil on the mentioned diabetic animal model and blood oxidative stress status.

First of all, I appreciate the authors for their valuable efforts to share these important findings with the readers. However, I have noticed some limitations that are not clearly highlighted in the limitations of the study. The authors mentioned the missing out of ezrin as a limitation of their paper. As a traditional application, life scientists usually use either  $\beta$ -actin or GAPDH as an internal control in Western blotting to compare the expression level of the protein of interest between groups. The authors used  $\beta$ -actin as an internal control. Literature reports that ezrin, radixin, and moesin (ERM) protein family members have cytoskeletal regulation activity by binding cytosolic actin to the plasma membrane. Several previous studies also reported disrupted  $\beta$ -actin as a result of oxidative stress.<sup>[2]</sup> Therefore, I believe that it would be better to choose GAPDH as an internal control. When I consider the microscopic findings, the immunohistochemical analysis of this study is very fascinating. For this reason, I appreciate the authors, but the limitations of this study can be enlarged when considering microscopic analyses. For example, besides the lack of data on ezrin, another ERM protein family-related cytoskeleton regulator protein merlin can be highlighted as a limitation of this study.<sup>[3]</sup> At least, I believe the findings of the merlin could be investigated to improve the output of this article.

When the biochemical analyses are evaluated, it is possible to reach similar limitations. The authors investigated the antioxidant potential of black cumin seed oil on the liver by considering MDA and GSH levels alone. The results of these analyses are very meaningful, but cytoskel-

eton alteration in oxidative stress is also regulated by  $\text{Ca}^{2+}$ -dependent mechanisms.<sup>[4]</sup> Therefore, better biochemical analyses could have been used to confirm the antioxidant potency of the black cumin seed oil in experimental diabetes.

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## References

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## Author's reply

Dear Editor,

I have been informed that you received a letter to the editor for our article entitled “Effects of black cumin seed oil on oxidative stress and expression of membrane-cytoskeleton linker proteins, radixin, and moesin in streptozotocin-induced diabetic rat liver”<sup>[1]</sup> from Seyda Nur Dagli. I express my gratitude to the author of the letter for her valuable contributions and criticism of our article. However, as the corresponding author, Until today, many experiments have been performed to investigate tissue-related pathogenic circumstances by investigating only two markers of oxidative stress, MDA and MPO.<sup>[2]</sup> In another published article, the authors investigated oxidative stress by considering tissue levels of MDA and GSH alone.<sup>[3]</sup> In another published article, we also investigated oxidative stress by considering only the MDA level in experimental animals under oxidative stress.<sup>[4]</sup> Although more analyses will increase the reliability of the results, it

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is possible to reach some information on the status of oxidative stress. However, I agree that the novelty of the article would have increased if the merlin level had been measured as stated by Seyda Nur Dagli.

In conclusion, while I thank Seyda Nur Dagli for her valuable contributions and recommendations, I still believe that the results of our study confirm the possible protective and antidiabetic activity of black cumin seed oil against oxidative stress. However, the results also indicate that the administered dose of black cumin seed oil was inadequate to protect hepatic cellular cytoskeleton structure.

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