**Dear Editor and Reviewers**

We highly appreciate all your insightful and very interesting comments on our manuscript. Thank you for taking the time and energy to help us improve the paper. All changes suggested by reviewer C were done and the manuscript was carefully revised according to the suggestions. So, we hope the reviewers and the editors will be satisfied with our responses to the ‘comments’ and the revisions for the original manuscript.

Thanks and Best Regards!

Yours Sincerely,

Ibrahim Arman

28/02/2020

Comments of reviewer C:

REPORT:

1. Original text - Molecular docking is a very important method in molecular biology and medicine design, such that docking- related papers have precipitously increased in https://www.ncbi.nlm.nih.gov/pmc, while the number of the papers were 2272 in 2004, it was reached to 11299 in 2018 in the website.

Suggested changes - Docking analysis is one of the mainstream in-silico methods used on a daily basis to predict binding modes of different proteins or DNA and small molecules. Key interactions between docked mole ules, binding site, binding conformation of molecules and estimated binding energy are some of the data provided by molecular docking analysis.( Give appropriate references where needed.)

Original sentence did not give potential reader any information about aim or usability of the molecular modeling as a method. In my opinion this should be changed.

Response: The original paragraph was replaced by sentences suggested by reviewer C and an appropriate reference was given.

2. Original text - The inconsistencies of the spectrophotometry and docking analysis results may be due to the differences in the methods by that the two analyses are performed. One is based on algorithms and scoring function (theory) and analyzes the interaction between DNA and drug while the other one is an experimental method that can be affected by some other ingredients and factors such as buffer and temperature.

Suggested changes – Both methods should give comparable results. One of the possible solutions to this problem is that model system that authors used, can be used to predict binding, but being a rather short DNA segment, cannot account for accurate binding constant prediction. To say that only reason for discrepancy in obtained results is difference in methods will automatically disqualify molecular docking result from being published (since experimentally obtained results have greater precision than those obtained by molecular docking procedure). Proper explanation is needed or **whole part with calculated binding constant should be deleted.**

Response: Whole part with calculated binding constant by docking system was deleted

3. Original text - The value of Kb for fluconazole is comparable to esculetin (an anti-cancer agent) (1.87×104 M-1) which binds to DNA.

Suggested changes – Authors should state if the esculetin Kb is calculated or experimentally obtained. Then they should proceed to explain why we should compare binding constants of esculetin and fluconazole, since their common structure is minimal.

Authors should address this properly or **delete whole sentence with esculetin.**

Response: Whole sentence with esculetin was deleted

4. Original text - In our opinion, the inconsistency was because of the following reseans; densitometer programs may be useful for comparing bands on the same gel, but not for comparing bands on different gels.

Suggested changes - This sentence is added in second revision of the manuscript. To my best knowledge, if used properly - densitometer programs are very accurate. There is no reason why results reported on two different gels cannot be compared.

**Authors should correct this statement or provide proper explanation.**

Response: The sentences of “densitometer programs may be useful for comparing bands on the same gel, but not for comparing bands on different gels” was deleted.

The mentioned part was revised as follows:

“The findings of our research were not consistent with the findings of Peng and her coworkers(Peng et al., 2018) who stated that fluconazole contributed to an increase of the DNA damage in vitro when complexed with iron or copper in the presence of hydrogen peroxide. In our opinion, the inconsistency was because they treated 0.1 pmol pBSSK plasmid DNA equal to 296400 µM bp of the plasmid with 50 µM of fluconazole. If they used the DNA concentration (bp) lower than fluconazole concentration, their results would be consistent with our results”.

Dear reviewer C we added the sentences regarding Peng work according to the suggestion of reviewer A in the second revision of the manuscript. In fact, we had before read the paper published by Peng but we could not use it before. Since in our opinion the part of that paper we could discuss in the discussion of our manuscript need to revise because:

1- In Figure 3D lane12 of the paper: the mixture of CuSo4 and H2O2 produces radical OH and it must have broken the DNA (Yokawa et al., 2011).

2- EDTA is a chelator and it can chelate Fe and Cu not radical OH present in the reaction mixture, therefore, it cannot stop the reaction of DNA breaking and conserve DNA against radical OH present in the mixture. On the other hand, the complex of Fe-EDTA can produce radical OH from H2O2 (Koppenol, 1985).

3- As it was shown in figure 3A Lane 5, FeSo4 + H2O2 produces radical hydroxyl and breaks DNA.

4. They treated 0.1 pmol pBSSK plasmid DNA equal to 296400 µM bp of the plasmid with 50 µM of fluconazole. The concentration of plasmid was more than the concentration of fluconazole. In our opinion, If they used the DNA concentration (bp) lower than fluconazole concentration the protection was provided by binding and covering DNA by the drug.

5. Original text - In conclusion, although fluconazole did not break double helix DNA even in the presence of H2O2 and ascorbic acid, it strongly bounds to double-stranded DNA. Fluconazole could protect DNA against radical hydroxyl originated from iron and H2O2 when its concentration was above DNA concentration. Therefore, fluconazole may make DNA fragmentation by binding to DNA.

Suggested changes – I find conclusion to be completely confusing. What I understood is:

- Fluconazole will bind to DNA but will not break DNA under stated conditions.

- Fluconazole may protect DNA under stated conditions.

- Fluconazole may fragment DNA by binding to it.

- I have a problem since “DNA fragmentation is the separation or breaking of DNA strands into pieces.” and in the first sentence authors state that breaking does not occur. Another problem is that earlier it the text authors

report that “hyperchromism derives from damage to the DNA, yet the absorbance value of the ct-DNA solution decreased at 260 nm and showed a hypochromic effect due to contraction of the double-stranded ct-DNA.”

– Thus, no damage was observed.

Please make the conclusion A more clear.

Response: The sentences of “DNA fragmentation is the separation or breaking of DNA strands into pieces” was deleted. The conclusion was revised as following:

In conclusion, fluconazole did not break double helix DNA even in the presence of H2O2 and ascorbic acid. According to the result of the spectrophotometric and docking analysis, the drug strongly binds to double-stranded DNA. In addition, fluconazole protects DNA against radical hydroxyl originated from iron and H2O2 when its concentration is above DNA concentration.

References:

KOPPENOL, W. 1985. The reaction of ferrous EDTA with hydrogen peroxide: evidence against hydroxyl radical formation. *Journal of free radicals in biology & medicine,* 1**,** 281-285.

LINDER, M. C. 2012. The relationship of copper to DNA damage and damage prevention in humans. *Mutat Res,* 733**,** 83-91.

PENG, C. A., GAERTNER, A. A., HENRIQUEZ, S. A., FANG, D., COLON-REYES, R. J., BRUMAGHIM, J. L. & KOZUBOWSKI, L. 2018. Fluconazole induces ROS in Cryptococcus neoformans and contributes to DNA damage in vitro. *PLoS One,* 13.

YOKAWA, K., KAGENISHI, T. & KAWANO, T. 2011. Prevention of oxidative DNA degradation by copper-binding peptides. *Biosci Biotechnol Biochem,* 75**,** 1377-9.