Reviewer’s report

Title: Impact of In utero Exposure to EtOH on Corpus Callosum Development and Paw Preference in Rats: Protective Effects of Silymarin

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Reviewer:

Level of interest: not specified

Advice on publication: Unable to decide on acceptance or rejection until the authors have responded to the compulsory revisions

The hypothesis tested in this study was that silymarin, a known hepatoprotective agent, may protect female rats from corpus callosum agenesis and altered lateralization (as measured by paw preference) attributable to prenatal ethanol exposure. Although the premise of the study is interesting the results are ambiguous and the discussion does not seem to add the necessary clarity.

Compulsory comments

1. The results indicate that there is no difference in the brain: corpus callosum ratio between experimental groups suggesting that on the basis of weight, ethanol did not cause general agenesis and silymarin was not protective. However, there were significant differences in corpus callosal weight between groups. Interestingly, the corpus callosum of the pair-fed control-offspring were significantly larger than those from any other group. Furthermore, the size of the corpus callosum from the ETOH exposed group and the chow control group were the smallest and were basically the same. Therefore the significance of weight as an indication of neural protection is not clear. Since weight was used one of two measures of callosal agenesis - it seems that it would be important for the authors to address these results in relation to the conclusion of the study. It would also be informative to provide the standard errors or standard deviations for the values in both Table one and Table two.

2. The observational results showing that silymarin prevents underdevelopment of the splenium found in ethanol exposed rats does support the assertions of the conclusion.

3. The finding that paw preference laterality is altered in ethanol exposed females (in which the left paw is favored) seems to be in agreement with the literature. The finding that silymarin both alone and in conjunction with ethanol causes the right paw to be favored instead of the 50% L - 50% R preference found in controls - does not suggest that silymarin is protective.

Interestingly, the left preference shown by the ethanol exposed females is correlated with incomplete
development of the splenium. Thus, it seems important that the authors present some references indicating the location of the fibers associated with paw preference in the corpus callosum.

4. The authors suggest that a possible explanation for the effects of both ethanol and silymarin on paw preference are (different) changes in sex steroids and their receptors (activities). It would therefore be of interest for the authors to discuss the differences in paw preference between male and female rats. Clearly, this study shows that in females there is no specific preference (left or right, in controls)

5. The discussion suggests to me that the authors are proposing that a possible mechanism of silymarin activity is the masculinization of the female corpus callosum by activating estrogen receptors prenatally - thus mimicking the male pattern of estrogen receptor activity. This would make sense if males have a right paw preference. It would be supported by the morphological data if paw preference neurons traverse the splenium. It would, therefore, be helpful if the authors would clarify their argument.

6. The second mechanism of silymarin activity proposed is binding to alpha-fetoprotein - and increasing availability of estrogen for neurodevelopmental processes. Again, this may support the protective effect of silymarin on splenium size, but does not present a convincing argument for changes in corpus callosal weight or paw preference. It would be helpful if the authors clarified this argument.

7. In the end, the conclusion that can be drawn is that splenium development is protectec by silymarin. Whether this is significant or not remains to be determined by further analysis of the ultra structure of splenia from animals in the experimental groups.

8. The references should be numbered.

Discretionary comments

Some corrections in wording-

Background

First sentence of first paragraph - delete the word "have" at the end of the sentence.

Fourth sentence of first paragraph, after children - - - prenatally exposed to ethanol.

Last sentence of first paragraph - after --- [5] noted - change "the" to "that"

In regard to the methods -

1. Was there a rationale for using females, rather than males, in this study?
2. What was the basis for the determination of the dose silybin used?
3. Might phosphatidylcholine, at the concentrations used in this study, be likely to have any effects on your results?
4. What is the rationale for discontinuing the experimental diets 5 days before birth?
5. A four day range in the age at weaning seems substantial. Have you found in past studies that this range has no effect on neural development or stress in your animals.

Results

1. Because the information in table two is important to the premise of the study it would be important to expound on the findings presented.

Discussion
There are a number of typos
1. Line 8 - "by" before [23] should be removed.
2. Line 9 - change cause to causes
3. Line 27 - "with the" is duplicated
4. The last sentence on page 10 is left hanging.

References
1. There are a number of typos in the references
2. The references should be numbered.

**Competing interests:**

None declared.