Reviewer's report

Title: Amelioration of galactosamine-induced nephrotoxicity by a protein isolated from the leaves of the herb, Cajanus indicus L

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Reviewer: Yoko Aniya

Reviewer's report:

Major comments
This article described that the 43kDa protein is protective against GalN-induced kidney toxicity and authors conclude that the protective action is due to antioxidant action of the protein in the kidney. Although they mentioned DPPH radical scavenging action of the protein in Background, the explanation of antioxidant action of the protein itself is not clear. So, its conclusion is not suitable. Also since GalN causes liver injury accompanying renal failure and the protein is hepatoprotective as previously reported, it is suggested that the protein protects against GalN-induced liver toxicity and consequently the renal failure is ameliorated. It may not be direct action of the protein to the kidney. Authors, at least, should discuss such points. The explanation of cellular metabolites (GSH, GSSG, total thiol) is not suitable, too. They measured GSH and GSSG levels. Their data show that GSSG is twice as GSH in control and GSH, which is decreased by GalN treatment, is increased markedly (2-fold) by the protein or vitE treatment. Thus it is assumed that the increased GSH scavenges ROS resulting in amelioration of the renal injury. The discussion should be considered. (Their discussion is not enough. They show general explanation of ROS but not GalN-induced oxidative stress. No explanation about marked increase in GSH by the protein etc).

Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)

Results
1) Figures should be summarized as follows: Fig 1 (creatinine), Fig 2(MDA) Fig.3 (A; SOD activity, B; Cat activity, C; GST activity; D;GR activity). Fig.4(A;GSH level, B;GSSG level, C; total thiol )
2) GSH and GSSG levels described as ug/mg should be expressed as umole/mg or nmole/mg.
3) Authors should explain what is total thiol. Does it include GSH, and other non-protein thiol?

Discussion
Line 8 ~26 (explanation of general oxidative stress) should be omitted. Instead, GalN induced oxidative stress mechanism in liver and kidney should be explained.

Also the followings should be considered.
1) The data show that GSSG in kidney is twice as GSH in normal condition(Fig7). In our knowledge, GSSG level is usually low (in liver, GSSG/GSH is about 1/50) and GSH is oxidized to GSSG by oxidative stress. Even if all GSH(15ug/mg) is converted to GSSG, the GSSG(64ug/mg) level in control is too high. It needs an explanation for kidney oxidative stress in relation to GSH/GSSG (or metabolism of GSH).

In addition GSH level is higher after the protein and vitE treatment than that of control. It seems that the protein/vitE stimulate GSH synthesis and the increased GSH seems to act as main antioxidant. It is better to explain them by citing other papers.
2) Also in total thiol, it needs to explain whether total thiol involves or not GSH and other non-protein thiol or protein thiols.
3) It is better to explain the 43kDa protein. In Background, authors described DPPH scavenging action of this protein. It should be explained how it act as...
antioxidant. Does the protein has many thiols? Is there any information of such protein from other plants?

4) As described in the background, GalN is well known toxin to cause liver toxicity which associates with the development of renal failure. It is therefore suggested that if the liver failure is recovered, then the renal failure is also recovered. Since the protein is hepatoprotective as seen in carbon tetrachloride etc.-induced liver injuries, the protein may ameliorate GalN-induced liver injury and consequently renal failure is reduced. It needs to discuss from such viewpoint.

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Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)

In Methods
1) How mice were sacrificed? (decapitation, under anesthesia?) It should be described.
2) Blood from heart was kept overnight. What temperature was used for the blood store overnight?
3) Animal treatment: What solvent (vehicle) was used for the protein and vitamin E preparation?
4) Kidney homogenates: How did authors homogenize the kidney? By glass homogenizer or polytron homogenizer? Buffer should be described as follows: 100mM KH2PO4 buffer to potassium phosphate buffer or KH2PO4-K2HPO4 buffer.
5) GST assay: What substrate was used? CDNB?
6) GR assay: molar extinction coefficient of 13,600 M-1 cm-1 is for what?
7) Total thiol: molar extinction coefficient of 13,600 M-1 cm-1 is for what?

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Discretionary Revisions (which the author can choose to ignore)

What next?: Unable to decide on acceptance or rejection until the authors have responded to the major compulsory revisions

Level of interest: An article of limited interest

Quality of written English: Acceptable

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:
'I declare that I have no competing interests.'