February 02, 2007

Professor Emma Parkin  
Associate Editor  
BMC-series journals

Dear Prof. Parkin,

Thank you very much for giving us the opportunity to revise our manuscript [MS: 6971848811237836]. I am submitting herewith the revised manuscript entitled, "Amelioration of galactosamine-induced nephrotoxicity by a protein isolated from the leaves of the herb, Cajanus indicus L" by Mahua Sinha, Prasenjit Manna and Parames C. Sil for publication in “BMC- Complementary and Alternative Medicine”.

For your kind information, I would like to mention that all the modifications have been made in the revised manuscript following the point-by-point comments and suggestions raised by the reviewers. Answers to reviewers have been provided below.

Kindly acknowledge the receipt of the revised manuscript. I look forward to receiving your decision at an early date.

Best regards

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Answers to Reviewers’ Comments
(Reviewer 1: Kenji Ishihara)

First of all I would like to thank the reviewer for his valuable comments and allowing us to revise the manuscript for consideration of publication in BMC Complementary and Alternative Medicine.

Major Compulsory Revisions

1- In addition to serum creatinine, we also measured another parameter, [urea nitrogen (UN)] to evaluate the GalN-induced renal damages. Following reviewers’ suggestion, it has been included in the revised manuscript.

2- We are in the process of performing histological studies on the GalN-induced renal abnormalities. The preliminary results under the experimental conditions following haematoxylin and eosin staining, however, showed no abnormalities in the kidney. As GalN induced renal failure seems to occur at the end stage of liver cirrhosis, the protective role of the protein against GalN induced renal damages is likely not to be its primary effect; probably the protein protects liver against GalN-induced toxicity and consequently the renal damage is reduced. Using a rat model, Anand et al reported similar result in their study [Ref no. 8 of our manuscript].

Minor Essential Revisions

1- All the typos including “glutsthione” have been corrected in the revised manuscript.

2- The typos of font style at texts in “Pre treatment with the protein” and “Post treatment with the protein” are corrected in the revised manuscript

Discretionary Revisions

Initially, we performed a dose-response analysis about the anti-renal damage of the protein (data not shown). Based on the result of that experiment, optimum dose (2mg/kg body weight) of the protein has been used in the present study.

Answers to Reviewers’ Comments
(Reviewer 2: Kiyohito Yagi)

First of all I would like to thank the reviewer for his valuable comments on our manuscript and allowing us to revise the manuscript for consideration of publication in BMC Complementary and Alternative Medicine.
Minor Essential Revisions

1. Page 4, line 3

The reference describing the DPPH radical scavenging activity of the protein has been changed from [16] to [31] in the revised manuscript.

2. Page 6, line 2-4

According to the reviewer, methods of GalN and protein administration have been written more clearly in the revised manuscript [Page 5, line 31 to Page 6, line 4].

The phrase “at an intraperitoneal dose of 800 mg/kg body weight for 3 days” means GalN was administered intraperitoneally at a dose of 800 mg/kg body weight once daily and the treatment was carried out for 3 days.

The phrase “the protein intraperitoneally at a dose of 2 mg/kg body weight for 4 days” means the protein was administered intraperitoneally at a dose of 2 mg/kg body weight once daily and the treatment was carried out for 4 days.

We have changed the statements in the revised manuscript.

Discretionary Revisions

3. We choose intraperitoneal route of administration for the protein. Intravenous administration of the protein to mice is very difficult. It requires considerable skill in locating the vein (in the tail) and the needle must be inserted into the vein (and not into the tissue that surrounds the vein). Also, absolutely no air can be accidentally injected, as this may cause air bubble blockage of blood circulation (and death of the animal). Initially we tried to perform the job but did not succeed. The reviewer is right; we think that the protein directly goes to bloodstream, reached to the kidney (either directly or indirectly) and expressed the effect.

4. Following the reviewers’ suggestion, we have discussed about the protein used in the study in the “Discussion” section of the revised manuscript.

Yes, there are proteins isolated from the medicinal plant possessing antioxidant activity. Details have been included in the revised manuscript [Ref 54-56]. Although details of the structural data of those proteins are not available, similar biochemical data have been described for those proteins. We have included that in the revised manuscript.

5. There are some errors in the calculations of GSH and GSSG values. The errors have been corrected in the revised manuscript. In addition, following to the suggestion of reviewer # 3, the units of these two parameters have been converted in “nmol/mg protein”. After necessary changes, practically there is no enhancement in the GSH levels in either the vitamin E or the protein treated groups compared to the normal controls.
Answers to Reviewers’ Comments  
(Reviewer 3: Yoko Aniya)

First of all I would like to thank the reviewer for his valuable comments on our manuscript and allowing us to revise the manuscript for consideration of publication in BMC Complementary and Alternative Medicine.

General
In addition to DPPH radical scavenging activity, it has been observed that the protein of our interest could also restore the activities of different antioxidant enzymes and attenuate the extent of lipid peroxidation. Since the detail mechanism of action of this protein is not known yet, we are modifying the language of the conclusion.

We agree with the reviewer that GalN induced renal failure appears at the end stage of liver cirrhosis. Our study protein possesses hepatoprotective activity and so amelioration of renal damage by this protein may not be the direct action of the protein to the kidney. Following reviewer’s suggestion such points have been discussed in the “Discussion” section of the revised manuscript.

Following reviewers comments on cellular metabolites, we checked the calculations of GSH and GSSG values and found that there are some errors in the calculations. In addition, following to the suggestion of reviewer, the units of these two parameters have been converted in “nmol/mg protein”. After all necessary changes, we found that practically there is no enhancement in the GSH levels in either the vitamin E or the protein treated groups compared to the normal controls.

Major Compulsory Revisions

Results

1) Following reviewer’s suggestion, figures have been summarized in the revised manuscript.

2) Following reviewer’s suggestion, units of GSH and GSSG levels have been expressed as nmol/mg protein in the revised manuscript.

3) Total thiol means total sulphhydryl groups in the system under measurements. It includes GSH and other non-protein thiols.

Discussion

Line8–26, Following reviewer’s suggestion, explanation of general oxidative stress has been omitted and GalN induced oxidative stress mechanism in liver and kidney has been explained in the revised manuscript [Page 11, line 30 to Page 12, line 22].
There are some errors in the calculations of GSH and GSSG values. The errors are corrected in the revised manuscript and the units of these two parameters are converted in “nmol/mg protein” according to the suggestion of reviewer. After correction the ratio of GSSG/GSH appears to about 1/50.

After necessary corrections it is found that GSH levels in protein and vitamin E treated animals are comparable to that of the normal control.

Total thiol means total sulfhydryl groups in the system under measurements. It includes GSH and other non-protein thiols.

Following reviewer’s suggestion, we discuss about the protein used in the study in the “Discussion” section of the revised manuscript. Partial sequencing of our study protein reveals that it contains some cysteine moiety. Yes, there are information of such proteins from other plants; references of other plant proteins possessing antioxidant activity are also included in the revised manuscript.

We agree with the reviewer. GalN is a well-known hepatotoxin and it was found that development of renal failure is associated with liver damage. Since previous findings reveal that the study protein possesses hepatoprotective activity, so it may ameliorate GalN induced liver injury and consequently renal failure is reduced. We discuss such points in the “Discussion” section of the revised manuscript.

Minor Essential Revisions

1) The mice of all the study groups were anaesthetized in ether and then sacrificed.
2) A temperature of 4°C was used for overnight blood storing.
3) Potassium phosphate buffer was used for protein and lipid (oil) was used for vitamin E preparation. Appropriate controls containing respective vehicles have also been used to compare the studied parameters.
4) The kidneys were homogenized by glass homogenizer and buffer composition is described in the revised manuscript.
5) In GST assay the substrate was CDNB.
6) In GR assay molar extinction coefficient of 13,600 M⁻¹ cm⁻¹ is for 5-thio-2-nitro benzoic acid (TNB).
7) In total thiol assay molar extinction coefficient of 13,600 M⁻¹ cm⁻¹ is for 5-thio-2-nitro benzoic acid (TNB).