

Too many hepatoprotective drugs and a little hepatoprotection

This is in response to the Research Communication entitled 'Hepatoprotection by *Phyllanthus amarus* and *Phyllanthus debilis* in CCl₄-induced liver dysfunction' (R. T. Sane *et al.*, *Curr. Sci.*, 1995, 68, 1243-1246) which describes the 'effectiveness' of *P. debilis* and *P. amarus* as hepatoprotective agents. The data are not presented properly and the conclusions drawn from them are partly misleading.

To evaluate hepatoprotection the data for CCl₄ + *P. amarus*-treated groups have to be compared with CCl₄-control group and not with normal control, and the statistical significance has to be determined between these groups. This has not been done. If there is complete hepatoprotection in CCl₄ + *P. debilis*- or *P. amarus*-treated groups, the values for serum enzymes and liver RNA shown in Tables 1 and 2 should not differ significantly between normal control group and CCl₄ + herbal drug-treated groups.

In Table 2, values for liver DNA and RNA are given. The levels of RNA decreased in the liver of CCl₄-treated animals compared to normal control. The RNA value for *P. amarus*-treated group (CCl₄ + *P. amarus*) is not statistically (significantly) different from that for CCl₄-control group. Furthermore, Table 2 has errors. All RNA values including the normal control value are marked with asterisks to indicate the level of significance. The SD for normal control group is 10.56 whereas that for group II is given as 0.7284. This is highly unlikely.

We have calculated the level of hepatoprotection in the herbal drug-treated groups based on the values given in

Tables 1 and 2 for serum enzymes and liver RNA and presented in Table A. In the case of *P. amarus*, out of 3 serum marker enzymes studied, only GPT activity shows hepatoprotection (63%). The level of alkaline phosphatase activity does not show any protection. GOT activity shows a marginal effect (36%). Further, CCl₄-induced decrease in liver RNA content was not significantly influenced by *P. amarus* treatment. With these observations it should not be concluded that *P. amarus* is effective as a hepatoprotective agent. A good hepatoprotective agent must influence almost all the relevant biochemical parameters more or less uniformly. If not, a plant drug control group has to be included to find whether the plant drug influences any specific parameters in normal rats.

In *P. debilis*-treated animals some hepatoprotection is seen. Even in this case, GOT and alkaline phosphatase activities show only around 50% protection and liver RNA level shows only 28% protection. The normal recovery from damage without herbal drug treatment in three more days (six days after CCl₄ treatment) is far better than *P. debilis* treatment for three days. As compared to the values in CCl₄-control on day 3, on day 6 the recovery from damage in serum enzymes and RNA is 60-80%.

Although the authors compared all the biochemical parameters with those of the normal control rats, the normal control rat liver histology figure is not given. It is better to show that, at least, for the benefit of those readers who are not familiar with liver histology. Some varia-

tions in the degree of histopathological changes can be seen in different rats of the same group and also, minor variations can be observed in different parts of the same liver. When these points are considered, among other things, the presented minor improvements in histopathological changes in the CCl₄ + herbal drug-treated groups as compared to CCl₄-control group may not represent good hepatoprotection.

Thus the reported data show, at best, a marginal hepatoprotective effect in *P. amarus*-treated rats. Considering all the parameters studied, the hepatoprotective effect of *P. debilis* is also below 50%. A reasonably good hepatoprotective agent should exhibit, at least, 60-80% hepatoprotection as seen in the case of normal recovery, 6 days after CCl₄-treatment (Table A). Drugs exhibiting at least 60-80% protective effect, preferably 80-100% protection, may be considered as hepatoprotective agents for treatment in crude forms after establishing their safety.

Although *P. debilis* showed moderate/slight hepatoprotection it should be noted that this effect of *P. debilis* was observed at a dose which gave maximum effect (0.66 g drug powder/kg body wt). The dose response effect is very important, and the data for the same are not given. The effect may not be seen or deleterious effect may be present at higher doses. Caution is required to use those drugs which do not give a normal dose response curve. Arbitrary dose fixation in herbal-drug treatment can result in the loss of moderate/small hepatoprotective effect of *P. debilis*.

Many common hepatoprotective medicinal plants are shown to have more than 60% hepatoprotection in most of the relevant biochemical parameters. However, comparison with published literature is sometimes difficult owing to the difference in the level of liver damage induced by various hepato-toxic agents (CCl₄, paracetamol, galactosamine, etc.) and in treatment protocol. To determine effectiveness as a hepatoprotective agent it is always better to compare with a hepatoprotective drug known for its efficacy and/or formulations such as Liv52.

We have studied both *P. debilis* and *P. amarus* and could not find substantive hepatoprotective effects in these herbs. We have observed relevant serum

Table A. Hepatoprotective effect of *P. amarus* and *P. debilis* in rats (% hepatoprotection)

Parameters	Groups			CCl ₄ (Normal recovery in 6 days)
	CCl ₄ -control	CCl ₄ + <i>P. amarus</i>	CCl ₄ + <i>P. debilis</i>	
GPT	0	63	98	60
GOT	0	36	45	65
Alk P	0	8	57	80
RNA (liver)	0	12	28	67

Percentage of protection was calculated as follows, assuming that there was no protection (100% damage) in CCl₄-control group

$$\text{Percentage of protection} = 100 - \left(\frac{100}{\text{Difference in the values between CCl}_4 \text{ control and normal control}} \times \left[\text{Difference in the values between CCl}_4 \text{ control and CCl}_4 \text{ + drug group} \right] \right)$$

enzymes, bilirubin, choloretic activity and liver histology to assess liver damage and protection from damage. In our studies on *P. debilis* we have found only 30–40% protection from paracetamol-induced elevation of serum enzymes and bilirubin at a dose of 1 g dry powder/kg body wt and we consider it as a small effect. In our screening we could detect *Phyllanthus* species which give 80–100% hepatoprotection regarding most of the biochemical parameters and liver histology.

There are numerous medicinal plants which have marginal or insufficient curative potentiality. Reporting them as effective or potent herbal remedies is misleading and dangerous to those who want to use them as remedies. Such reports will increase the numbers in the long list of hepatoprotective herbal drugs but not provide hepatoprotection to the patients.

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R. T. Sane et al.'s reply

The publication presented the preliminary findings of our studies on the two *Phyllanthus* spp. We have not claimed *Phyllanthus debilis* to be the best hepatoprotective agent. The publication was initiated because our surveys indicated that all species of *Phyllanthus* are being used as hepatotonics with no species considerations. The study has brought out clearly the species-dependent variation in the hepatoprotective action and highlights the need to be very selective in their use.

Statistical comparison of treated groups with the normal control group is justified because the extent of recovery after treatment will be better evaluated with normal liver rather than a damaged one, as suggested by A. Subramoniam (AS). Even if such a comparison is made, no significant changes will occur in our evaluation. We have not claimed complete hepatoprotection in our findings.

We regret inadvertent error of marking

the normal values also with asterisks. The SD of group II is 0.7284 and has been reported correctly. Several papers have been published, which clearly show that *P. amarus* is not a good hepatoprotective agent. The suggestion about adding plant control group is good. We appreciate the analysis of percentage hepatoprotection for each of the experimental groups. It is evident that *P. debilis* is a better hepatoprotective agent than *P. amarus*. The recovery seen in normal recovery group is after a period of six days, whereas the recovery in plant-treated groups is after three days. Average recovery seen for each group from AS's analysis is given below:

CCl ₄ control	CCl ₄ + <i>P. amarus</i>	CCl ₄ + <i>P. debilis</i>	Normal recovery
00%	30.0%	57.0%	68%
	after 3 days	after 3 days	after 6 days

It is very clear that the rate of recovery by natural regeneration is slower than the *P. debilis*-treated group. Besides, published papers indicate that complete recovery after CCl₄-induced damage is achieved only after two weeks. We do agree that there is better recovery of RNA levels in natural recovery group.

The photograph of normal liver histology was not provided since the structural characteristics needed no special mention. After scanning the entire liver area, the most representative area was reproduced and we do not consider the improvement seen in them as minor but, quite significant.

The dose of the plant slurry was not fixed arbitrarily. We have carried out a dose-response study, after which the

specific dose of *P. debilis* was fixed. The details of these findings could not form part of a short research communication. We agree that dose fixation is very important to avoid doses of deleterious effects or no effect. In fact, we have also carried out toxicity studies on these plant slurries. In the light of the findings from these studies we found the dose of 1 g/kg to be on the higher side and mildly toxic. This could be the reason why experiments with *P. debilis* could not provide satisfactory results.

It is also to be noted that various hepatotoxicants cause hepatic injury in different ways. Therefore, evaluation of hepatoprotective or hepatocurative actions need to be carried out with careful analysis to avoid over-interpretation. We are eagerly awaiting the publication on screening experiments by AS, where a *Phyllanthus* sp. is said to be identified, with better hepatoprotective action.

We have already initiated further studies on other *Phyllanthus* spp. and plan to use different hepatotoxicants. We have also carried out electron microscopic studies and have evaluated several other biochemical parameters. We are confident of publishing our findings soon. We still believe *P. debilis* to be a good hepatotonic.

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Cryopreservation of epididymal spermatozoa

In 1949, Polge *et al.*¹ in England made a serendipitous discovery that glycerol protected fowl spermatozoa from the otherwise lethal effects of freezing. In 1953, Bunge and Sherman² demonstrated for the first time that frozen and thawed human spermatozoa could result in pregnancy and the birth of normal babies. All subsequent published reports have

confirmed the cryopreservability of ejaculated seminal spermatozoa. The first semen bank in India was started at Apollo Hospital in January 1988.

Obstructive azoospermia is an important cause of male infertility. Surgical correction of obstruction yields poor results except when single tubular anastomosis is undertaken³. In some cases, surgical