enzymes, bilirubin, choleretic 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* sps 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 + *P. amarus*	CCl_4 + *P. debilis*	Normal recovery
00%	30 0% after 3 days	57 0% after 3 days	68% 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 CCl_4-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* sps. 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 Sherman[2] 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 anastamosis is undertaken[3]. In some cases, surgical

correction is not possible (as in vasal-aplasia). However, in most of these men, a good number of spermatozoa with variable motility could be obtained by epididymal aspiration. It is not always possible to use the spermatozoa obtained by epididymal aspiration for Assisted Reproductive Technology as this requires an additional expense and simultaneous preparation of the wife. Therefore it was decided to try and cryopreserve epididymal spermatozoa obtained from caput epididymis (in men undergoing scrotal exploration) so that this could be used at a later date if necessary.

The subjects for the study were three men undergoing scrotal exploration for obstructive azoospermia in 1991. Informed consent was obtained from all these men. Epididymal spermatozoa were aspirated from caput epididymis into 1 ml of Earle's balanced salt solution. The prefreeze spermiogram was done. This was then mixed with an equal volume of Matheson Carlborg–Gemzell medium (cryoprotectant[4]) and cryopreserved in a biological freezer at $-70^{\circ}C$.

Good motile spermatozoa were obtained from all the three men from caput epididymis. The sperm concentration prefreeze were twelve million, ten million and thirty-five million per ml with 30%, 40% and 60% progressively motile spermatozoa. Vaso epididymal anastomosis was done on two men. One man had vasal aplasia. One of these men, two months later, showed a sperm concentration of eighty million per ml with 30% motility and his wife was pregnant a month later. She has since delivered a baby. The other man had no spermatozoa at the first follow up, a month later, and has not reported back since then. As these three men were not keen on Intra Uterine Artificial Insemination Husband or ART for their wives, the spermatozoa so obtained from the caput were frozen with their consent. After one month the sample was taken out and thawed and it was found that the spermatozoa in all the three samples had still maintained 40–50% of their prefreeze motility.

Surgical correction of obstructive azoospermia generally yields poor results (20%). Unless and until experience is gained with single tubular anastomosis, the results of vaso epididymal anastomosis are not likely to improve. However, many centres have good experience with GIFT/IVF by conventional means or by Micro Insemination and Sperm Transfer (MIST), Sub-Zonal Insemination (SUZI) or Intra Cytoplasmic Sperm Injection (ICSI). For all these techniques, the number of spermatozoa required is far less than for *in vitro* fertilization. Therefore, while attempting vaso epididymal anastomosis for obstructive azoospermia, if the couple are prepared, one could also undertake IUAIH or ART. If, however, the couple are not prepared, one could attempt to cryopreserve the epididymal spermatozoa and use the cryopreserved spermatozoa for ART later if the surgical correction were to be unsuccessful.

All mammalian cells are not cryopreservable. It is not known what offers the spermatozoa the cryopreservable capacity. The fact that epididymal spermatozoa could be cryopreserved indicates that human spermatozoa do not require the presence of seminal constituents for being cryopreserved.

Epididymal spermatozoa which are mature, motile and capable of fertilization are also cryopreservable. It is suggested that all attempts at surgical correction of obstructive azoospermia should be accompanied by an attempt at IUAIH/ART or at least cryopreservation of epididymal spermatozoa so obtained.

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Cytokine antagonism by active vaccination

The soluble mediators, known as cytokines, regulate immune response as well as effector phase of immune reactions. They are characterized by functional pleiotropy and redundancy and act as a 'network'. They are required for a number of normal reactions in the body but produce pathological changes when they are present at the wrong place and in wrong concentrations. Their role in a number of diseases has generated keen interest in the cytokine antagonists and their potential therapeutic use. The cytokine antagonists are nonpeptide molecules, soluble receptors, IL-1ra/mutated cytokines and autoantibodies[1]. Besides these, the adverse effects of cytokines have been neutralized successfully by passive immunization of animals by anticytokine-antibodies[2–6]. On the other hand, the non-neutralizing type of antibodies contribute to a positive therapeutic response by better targeting of the cytokines to the appropriate cells[7,8]. We have successfully used a novel approach to actively immunize mice with a dengue virus-induced cytokine, the mouse Cytotoxic Factor (mCF), which acts as 'pathogenesis-related protein', to protect against the pathological effects[9]. We wish to draw attention to this potentially therapeutic approach of 'anti-disease vaccination'.

During dengue type 2 virus (DV) infection of mice, a unique cytokine mCF is produced by T lymphocytes. mCF is a 43 kD molecule on native PAGE and has an isoelectric point of pH 6.5. The sequence of 19 amino acids of the *N*-terminus of mCF (compared at Gen Data Base, Distributed Information Centre, Indian Institute of Science, Bangalore) differs from those of other cytokines and DV-specific proteins[10]. Northern blot tests done with the oligonucleotide probes derived from this sequence shows the presence of mRNA for mCF in the spleen cells of DV-infected mice. The cDNA library of mCF has been constructed in λgt 11 and expressed in *Escherichia coli*