Corrigendum

1. Initially survivality of splenic lymphocytes in presence of three doses (5, 10 & 25 µl) of ethanolic leaf extract of *Eupatorium adenophorum* (EEA) was observed *in vitro* at several hours as they are in Fig. 4. At each hour for different doses three cultures were maintained in an experiment and the experiment repeated thrice. Thus, in the final figure, each point represents nine readings. The data are now appended herewith (marked as Fig. 4A as continuation of Fig. 4 in the thesis). The percentage of cell viability was found to be at reasonable level with 25 µl dose. In view of EEA's efficacy at 25 µl dose and to keep the amount of vehicle on the lower side, further higher doses were not preferred. That is why the 25 µl dose was used in subsequent experiments.

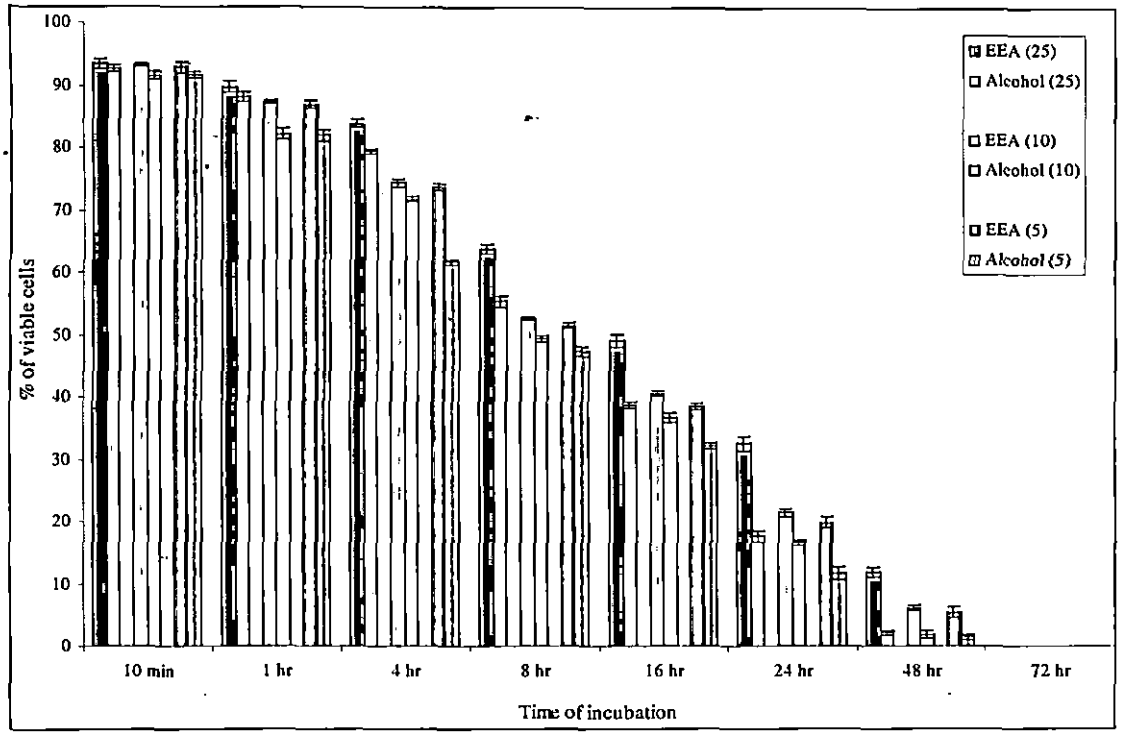

Fig. 4A: Survivality of lymphocytes *in vitro* in presence and absence of EEA. Results are expressed as mean ± SD. Numbers in parenthesis indicate doses in µl.

As the 25 µl dose was found effective *in vitro* the same was maintained for *in vivo* experiment, and it was found to be non-toxic for the animals and also activate

lymphocytes *in vivo*. Two different doses for *in vivo* and *in vitro* experiment might not allow discussion of *in vivo* and *in vitro* studies at a time.

It is not possible to carry out all the experiments over the years with the same batch of extract. It is expected that batch to batch there would be certain minimal variations in the constituents. We tried to maintain a dry weight equivalence for different batches of EEA extract. 1 ml of EEA after each batch of extract preparation was evaporated to dryness under reduced pressure (Rotary Vaccum, EYELA, Japan) at 55^0 C and the dry weight was in the range of 0.529±0.019 mg. This is mentioned under Preparation of ethanolic leaf extract of *Eupatorium adenophorum* (EEA) in Materials and Methods in Page 10. Dry weight equivalence indicates amount of bulk material in different batches of extract and there might be subtle variation in principal compounds. However, there were not much significant differences in the results from experiment to experiment and the final data represents average of all the experiments.

2. In the present thesis, the figures and tables have been formatted with running text in the result section. The explanatory note for each figure and table were in the text. That was not included in the legends just not to be repetitive.

3. Fig. 3 in page 30 denotes total count of WBCs in blood. It is mentioned in Page 29 that the effects of the extract on blood parameters have been studied in this section.

The proliferative response of the total WBCs on treatment with EEA seems to reflect promotional effect of the extract towards the leukocytes. This has been mentioned in Page 30 of the result section, and this proliferative activity of WBC advocated probable immunostimulatory activity of the extract and encouraged further evaluation of EEA as immunomodulatory agent (mentioned in Discussion, Page 63, Para 2, Line 1).

The effect of EEA on hematological parameters was studied on different days after injecting (i.v.) 25 µl EEA or ethanol in mice only once on '0' day (Fig. 1-3).

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4. The information about number of cultures or animals used in each experiment has been stated under 'Statistical analysis' in Materials and Methods, in page 28 – "Triplicates in experimental and control set were maintained (for each point) in each experiment. An experiment was repeated thrice or more. Results are expressed as Mean±SD of *n* observations". Thus, for both *in vitro* and *in vivo* experiments, data at each point represent at least 9 readings from cultures or animals.

5. The cells in culture medium only (untreated cells) gradually die off in course of culture. Alcohol control was always maintained as alcohol was the vehicle for leaf extract of *E. adenophorum*. So the data of untreated cells were not included in Fig. 4, page 31 to keep the diagram focussed on the effect of the extract. However the results of untreated cells at 16, 24 and 48 hrs are included here in Fig. 4B. In our experience, the percentage of survival of unactivated (without any treatment) splenic lymphocytes is always poor.

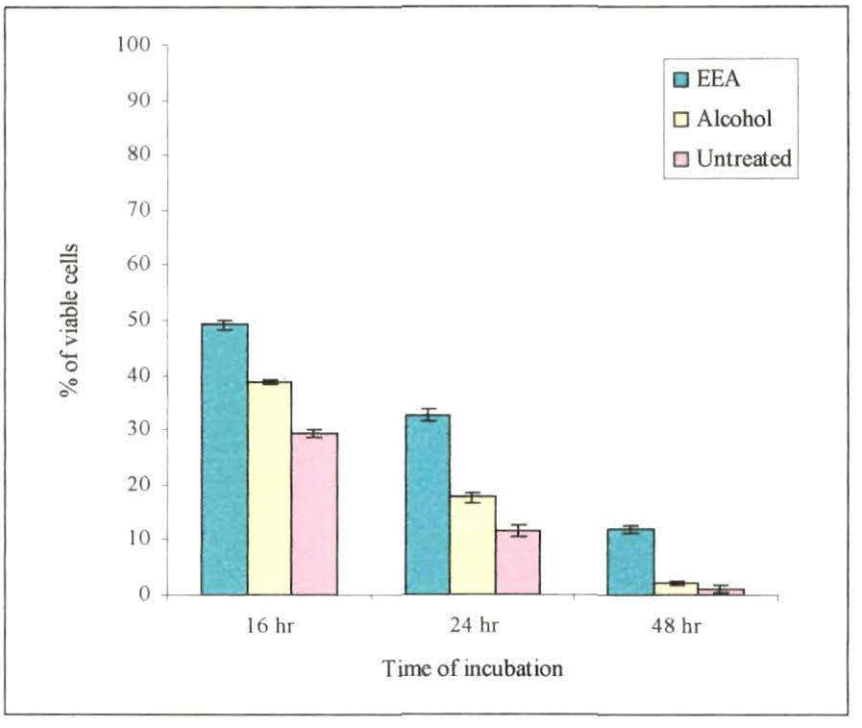

Fig. 4B: Survivality of lymphocytes *in vitro* treated with EEA, alcohol and without any treatment in medium only. Results are expressed as mean ± SD.

6. Page 32, Fig. 5: One of the main objectives of the present study was to judge the immunomodulatory property of EEA, and significantly higher percentage of blastogenic transformation of both T and B lymphocytes with EEA over ethanol control was found. Experiments with several conventional mitogenic polyclonal stimulators for murine lymphocytes were carried out in our laboratory earlier (see in Reference Chakravarty and Chaudhuri, 1983; Chakravarty and Maitra, 1983; Chaudhuri and Chakravarty, 1983a; Chaudhuri T.K. and A.K. Chakravarty. (1981). *J. Ind. Inst. Sci.* 63(C): 149-156; Maitra U.K. and A.K. Chakravarty (1982). *Current Sci.* 51: 1012-1015.). The stimulatory property of any mitogenic substance is usually measured in reference to the vehicle control. There is a note about untreated lymphocytes (i.e. in culture medium only) in point 5. The data of untreated splenocytes is not provided because lymphocytes without stimulation are not supposed to undergo blastogenic-transformation.

7. Page 33, Fig. 6: Our laboratory has remained busy for last one decade to characterize immunotherapeutic activities of bioactive natural products such as turmeric (*Curcuma longa* Linn.), *Eupatorium adenophorum* and *Leucas aspera*. The efficacies of ethanolic extracts from these agents were always compared with that of ethanol as vehicle control. Many a times, ethanol was found to have some stimulatory effect compared to untreated control at initial hours only and the effect did not continue too long: That is also reflected in the measure of DNA synthesis in lymphocytes in the present study.

It seems that the maximum level of stimulation for DNA synthesis with EEA treatment was achieved by 16 hrs and the proliferation index continued further. This has been written in Result section, page32, para 2.

8. Page 34, Table 1: Cell cycle analysis by FACS reading was carried out at Central Regional Instrument Facilities, Bose Institute, Centenary Building, Kolkata. The software

of this machine (FACS, Caliber, Beckton Dickinson) has counted the cells in four different phases – G_0, G_1, S and G_2-M (denoted as M1, M2, M3 and M4 in Fig. 7, Page 35). The G_0 phase signifies the cells in quiescent or apoptotic stages as stated in Page 34, Para 1.

9. Page 35, Fig. 7: The data in Fig. 7 were automatically generated by the FACS machine. At 16, 24 and 48 hrs the cells were pooled from respective cultures and adjusted to 10^6 living cells per ml for FACS reading. It is likely even after two washes some of the dead cells from the culture got included in the counting sample. The first peak includes dead cells and quiescent cells as indicated in point 8.

10. Page 36, Fig. 8: The inhibitory effect of topical application of EEA and alcohol on DTH is comparable for first two days. From third day onward EEA was slightly better in inhibition over control and reached a significant level by 8^{th} and 9^{th} day.

11. Fig. 9: Higher dosage (50 µl) of DNFB induced a much higher level of DTH swelling in comparison to that of lower dosage (25 µl) of DNFB (Fig. 8). Perhaps inhibitory effect of alcohol was not discernable with a much bigger DTH swelling. But EEA could show once more its effectiveness in inhibition of DTH.

12. Page 41, Fig. 14: EEA's ability to stimulate $CD4^+T$ lymphocytes significantly, is mentioned in result section of page 41 and in Discussion in page 64, para 2, line 9. It is

fairly indicated that by property EEA is acting as a mitogen but this has not been mentioned overtly.

13. Page 41, Fig. 13: The differential count of WBCs at DTH site shows EEA could effectively inhibit recruitment of monocytes, neutrophils and eosinophils besides promoting lymphocyte number. The possible explanation for these events have been discussed in page 64, para 3. Further, the discussion is continued in page 65 to tell about regulation of participatory cytokines in inflammation.

14. Page 47, 48, Fig. 21: The results of blastogenic transformation (Fig. 5) showed that EEA activates T cells. It is known that polyclonal activation of T cells can generate cytotoxic cells toward tumor target cells (mentioned in Introduction, page 5, para 3, line 5 and Discussion, page 71, para 2, line 4).

In our experience not necessarily the percentage of cytotoxicity is always proportional to the increment of ratio of effector to target cells. The data were presented the way we observed in Fig. 21. We did not want to enter into many suppositions to explain the results.

15. Page 47, Fig. 20: A sudden increase in tumor size from 75 to 89 days in EEA treated mice is notable indeed. The trend of increment actually starts from 61 days onward. Possibly regular dose of 5 µl EEA for topical application on the growing tumor is not sufficient to restrict the tumor growth for all the time, although it was effective in initial phase.

The focus in this study was to analyze the effect of EEA on tumor growth in reference to alcohol control. We did not keep the set without any treatment in the present experiment as we are aware about the growth of tumor bearing mice in the similar set maintained in connection of experiments testing efficacy of turmeric performed earlier in our laboratory, and not to kill the animals unnecessarily this time. However, the mice without any treatment survived for 60 to 65 days bearing tumor of 2.198 to 2.322 sq.cm.

16. Page 49, Figs. 22 and 23: PCR protocol followed here is a basic one adopting the mRNA phenotyping procedure and the principle of Real Time PCR is also based on it. We had the set up and chemicals in our laboratory to continue with the usual PCR. It is true that Real Time PCR would possibly provide quantitation of gene expression on the Ct value. (Kindly refer to the galley proof of the paper accepted for publication in "Evidence Based Complementary and Alternative Medicine" (eCAM), Oxford University Press, at the end of the thesis.)

17. Page 52, Fig. 24: PFC assay has been carried out with splenocytes from mouse injected with EEA or ethanol 1 day prior to primary immunization (Materials and Methods, page 23). There were no significant variations between the sets in both primary and secondary immune response; 'p' values calculated are greater than 0.5 in all cases.

18. TLC separation of the total extract showed a major UV active band at Rf 0.54, indicating the major constituent of the total EEA extract. This particular band was further analyzed for chemical characterization and the band was found to be terpenoid. It is true that there are certain other components in the total extract and characterization of those will initiate a new study in future.

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