BMC Complementary and Alternative Medicine

Research article

Effect of a homeopathic drug, Chelidonium, in amelioration of p-DAB induced hepatocarcinogenesis in mice

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Published: 10 April 2002

BMC Complementary and Alternative Medicine 2002, **2**:4

Received: 7 December 2001

Accepted: 10 April 2002

This article is available from: http://www.biomedcentral.com/1472-6882/2/4

Abstract

Background: Crude extracts of *Chelidonium majus*, and also purified compounds derived from crude extracts of this plant, have been reported to exhibit anti-viral, anti-inflammatory, anti-tumor and anti-microbial properties both *in vitro* and *in vivo*. Chelidonium is a homeopathic drug routinely used against various liver disorders including cancer in humans. Two potencies of Chelidonium (Ch-30, Ch-200) have been tested for their possible anti-tumor and enzyme modulating activities in liver and anti-clastogenic effects during p-DAB-induced hepatocarcinogenesis in mice compared to suitable controls.

Methods: Several cytogenetic and enzymatic protocols were used at three fixation intervals; at 60 days, 90 days and 120 days of treatment. Different sets of healthy mice were fed: i) hepatocarcinogen, p-DAB plus phenobarbital (PB), ii) only PB, iii) neither p-DAB nor PB (normal control). One set of mice fed with p-DAB plus PB was also fed Ch-30 (iv) and another set Ch-200 (v). All standard currently used methods were adopted for cytogenetical preparations and for the enzyme assays.

Results: All group (i) mice developed tumors in liver at all fixation intervals, while none of group (ii) and (iii) mice developed any tumors. About 40% mice in group (iv) and group (v) did not show tumor nodules in their liver. Feeding of Chelidonium to group (iv) and (v) mice reduced genotoxic effects to a significant extent ($p < 0.05$ to $p < 0.001$).

Conclusion: The homeopathic drug Chelidonium exhibited anti-tumor and anti-genotoxic activities and also favorably modulated activities of some marker enzymes. Microdoses of Chelidonium may be effectively used in combating liver cancer.

Background

Chelidonium majus L. (Papaveraceae) is a plant of great interest for its use in various diseases in European countries and in Chinese herbal medicines. Crude extracts of various parts such as the root, shoot and leaves have been reported to have several isoquinoline alkaloids, such as, sanguinarine, chelidonine, chelerythrine, berberine and coptisisine. Both crude extracts of *C. majus* and purified compounds derived from it have been reported to exhibit interesting anti-viral, anti-inflammatory, anti-tumor and anti-microbial properties both *in vitro* and *in vivo*[1–3]. Besides, inhibitory effect of *Chelidonium majus* herb extract has been reported on growth of keratinocytes in human, and on lipoxygenase activity in mice [4] while stimulatory

effect has been reported on bile acid independent flow in isolated perfused rat liver [5].

In the homeopathic mode of treatment, various micro doses (potencies) of Chelidonium herb extract are routinely used against several forms of liver disorders, including liver cancer [6] with good effect. But, to our knowledge, whether ultra-low doses of *Chelidonium majus*, namely, Chelidonium-30 (Ch-30) and Chelidonium-200 (Ch-200), could also have similar anti-tumor or anti-genotoxic activities had not been experimentally tested so far in mice *in vivo*. The present investigation was therefore undertaken primarily to examine if Ch-30 and Ch-200, prepared as per homeopathic procedure, could show i) antitumor activity in liver, ii) anti-clastogenic effect in bone marrow cells, iii) protective/repair ability on sperm heads, and iv) ameliorating effects in the activities of some marker enzymes like acid and alkaline phosphatases, and peroxidase in various tissues during azo dye induced hepatocarcinogenesis in mice.

Materials and methods

Materials

An inbred strain of Swiss albino mice (*Mus musculus*), reared and maintained in the animal house of the Department of Zoology, (under the supervision of the Animal Welfare Committee), Kalyani University, served as materials for the present study. Mice were provided food and water *ad libitum*. The food was made of wheat, gram and powdered milk without any animal protein supplement. With due permission from the Animal Welfare Committee, Kalyani University, which also oversees ethical issues of animal experimentation, the present investigation was undertaken. A group of 25 healthy mice weighing between 25–30 grams were used for each of the three long term fixation intervals viz. 60, 90 and 120 days. Each group was further divided into five different sets consisting of five mice each. The first set of mice were allowed normal low protein diet mixed with 0.06% p-DAB (Sigma, D-6760), a known "initiator" of hepatocarcinoma, and water *ad libitum*, till 30 days after which the water was replaced with 0.05% aqueous solution of PB, a known "promoter", till they were sacrificed. The second set of mice were provided with low protein diet without p-DAB and 0.05% aqueous solution of PB instead of pure water after one month as in group (i) till they were sacrificed. For the third set of mice the low protein diet was neither mixed with p-DAB nor water was replaced with PB. The third set served as negative control. The fourth set of mice were given p-DAB and PB in the same way as that of the first group but were also fed 0.06 ml of stock solution of the drug-Ch-30 thrice a day (6 A.M, 12 Noon, 6 P.M) from first day onward of p-DAB feeding, for seven days, and then twice a day (6 AM, 6 PM) till they were sacrificed. In the fifth set of mice the feeding of p-DAB, PB and Ch-200 followed the same manner as that of the fourth set of mice, except that the drug was fed twice a day (6AM, 6PM) all along till they were sacrificed.

Preparation of the potentized homeopathic drug

The two potencies of Chelidonium, procured from "HAPCO", 165, Bipin Behari Ganguli Street, Kolkata, were prepared as per the standard procedure of homoeopathic drug preparation. The dry drug material of *Chelidonium majus* (whole plant) was extracted in 44% ethyl alcohol (i.e. the "mother tincture"). 1 ml of the mother tincture was subsequently diluted with 99 ml HPI approved solvent (IP 96/HPI grade ethyl alcohol) and "succussed" 10 times to make the potency 1. The potency 2 was similarly made by diluting 1 ml of potency 1 with 99 ml of ethyl alcohol and giving 10 jerks, and the procedure was repeated to get the microdoses of Ch-30 and Ch-200.

Preparation of stock solution of the drug

1 ml each of Ch-30 and Ch-200 was finally diluted separately with 20 ml of double distilled water to make the stock solution of Ch-30 and Ch-200, respectively.

Feeding procedure and dose

Each mouse was fed 1 drop (0.06 ml) of either Ch-30 or Ch-200 from the stock solutions at a time with the aid of a fine pipette.

Cytogenetic assay

Mice were intra-peritonially injected with 0.03% colchicine @ 1 ml/100 gm body weight 1 hr and 30 min before sacrifice. Marrow of the femur was flushed in 1% sodium citrate solution at 37°C and fixed in acetic acid/ethanol (1:3). Slides were prepared by the conventional flame drying technique followed by Giemsa staining for scoring bone marrow chromosome aberrations. A total of 500 bone marrow cells were observed, 100 from each of 5 mice of a set.

For micronucleus (MN) preparation, a part of the suspension of bone marrow cells in 1% sodium citrate was smeared on clean grease free slides, briefly fixed in methanol and subsequently stained with May-Grunwald followed by Giemsa. Approximately 5000 bone marrow cells, comprising both polychromatic erythrocytes (PCE) and normochromatic erythrocytes (NCE) were scored and the ratios between PCE and NCE were calculated.

The mitotic index (MI) was determined from the same slide which was scanned for MN. The non-dividing and dividing cells were recorded and their ratios ascertained.

For sperm head anomaly (SHA), the epididymis of each side of mouse of both (control and treated) sets was dissected out and its inner content squeezed out into 10 ml

of 0.87% normal saline separately. It was made free of fats, vas deferens and other tissues. The content was thoroughly shaken, filtered through a silken cloth and dropped on grease free clean slides. The slides were allowed to air dry and then stained by dilute Giemsa (1 ml Giemsa in 10 ml distilled water).

Biochemical assays

Mice were sacrificed and their liver, spleen and kidney were quickly isolated. The tissues were homogenized with cold 0.87% normal saline, followed by centrifugation at 3000 g for 20 minutes in cooling centrifuge (C24, Remi Instruments). Before carrying out the enzymatic estimations the quantitative estimation of total protein was made by the method of Lowry *et al*[7]. To 0.1 ml of the sample, 0.9 ml of 0.1 (N) NaOH was added. Then 5 ml of alkaline solution was added to the sample solution followed by 0.5 ml of Folin-Phenol reagent and after 30 minutes the extinction was read at 750 nm against appropriate blank in spectrophotometer (Shimadzu, Double beam Spectrophotometer UV-180, Japan).

Estimation of Lipid Peroxidase

The lipid peroxidation was estimated from the supernatant by the method of Buege and Aust [8]. 1 ml of sample (homogenate containing 0.1–0.2 mg of protein) was mixed thoroughly with 2 ml of TCA-TBA-HCl (15% w/v TCA and 0.375% w/v TBA in 0.25 N HCl,). The absorbance of the sample was determined at 535 nm in a double beam spectrophotometer against a suitable blank The malonaldehyde concentration of the sample was calculated by using extinction coefficient of 1.56×10^5 $M^{-1}cm^{-1}$.

Estimation of Acid and Alkaline Phosphatases

For the study of acid and alkaline phosphatases method of Walter and Schutt [9] was followed. For acid phosphatase, to 0.2 ml tissue homogenate 1 ml of acid buffer was added to make the volume 1.2 ml. It was mixed and incubated at 37°C for 30 minutes. Then 2 ml of 0.1 (N) NaOH was added. The absorbance was measured at 405 nm against a blank. Then the activity of acid phosphatase was calculated as mM phenol liberated per mg of protein after 30 min of incubation at 37°C using suitable standard curve.

For alkaline phosphatase activity the 0.05 ml homogenate was mixed with 2 ml alkaline buffer so that the volume always stood at 2.05 ml. It was incubated at 37°C for 30 minutes, then 10 ml of 0.05 N NaOH was added and the absorbance was measured at 405 nm against a blank. The activity of alkaline phosphatase was calculated as mM phenol liberated per mg of protein after 30 min of incubation at 37°C using suitable standard curve.

Statistical analysis and scoring of data

The significance test between different series of data was conducted by student's t-test. During preparation of slides for cytogenetical observation and biochemical estimation of the different enzymes, the "observer" was kept "blinded" in order to remove any "bias" in observation and to keep uniformity in scoring data of both treated and control sets of mice.

Results

Out of the total number of 45 mice fed with p-DAB plus PB and sacrificed at three fixation intervals, livers in some 27 mice showed distinct sign of tumor formation in the form of pale reddish multiple nodules (some other relevant histo-pathological data of liver in p-DAB+PB treated and untreated control mice also provided in table-1), while the remaining ones did not develop such nodules. All mice fed p-DAB plus PB but no homeopathic drug developed tumorous nodules in liver and also had appreciably enlarged spleen. However, every 2 out of 5 mice that received either Ch-30 or Ch-200 alongside p-DAB plus PB did not show tumorous nodules, although they had slightly enlarged spleen. Thus, in the present experiment there was an overall 40% decline in the occurrence of tumors owing to the treatment of Ch-30 and Ch-200.

Table 1: Showing some salient changes in liver histology of p-DAB+PB treated mice (showing tumors) as compared to untreated controls

p-DAB + PB treated series showing tumors	Normal control series without tumor
In majority of cells, more than one nucleus per cell present, some nuclei very large	Generally one nucleus per cell present
Vacuolated cytoplasm and loss of distinct cell outline in most cells,	Cytoplasm not vacuolated
The number of hepatocytes, bile and duct cells increased	Number of hepatocytes bile and duct cells relatively few
The spaces between adjacent cells not well-defined	Cell outline distinct and spaces between adjacent cells well-defined

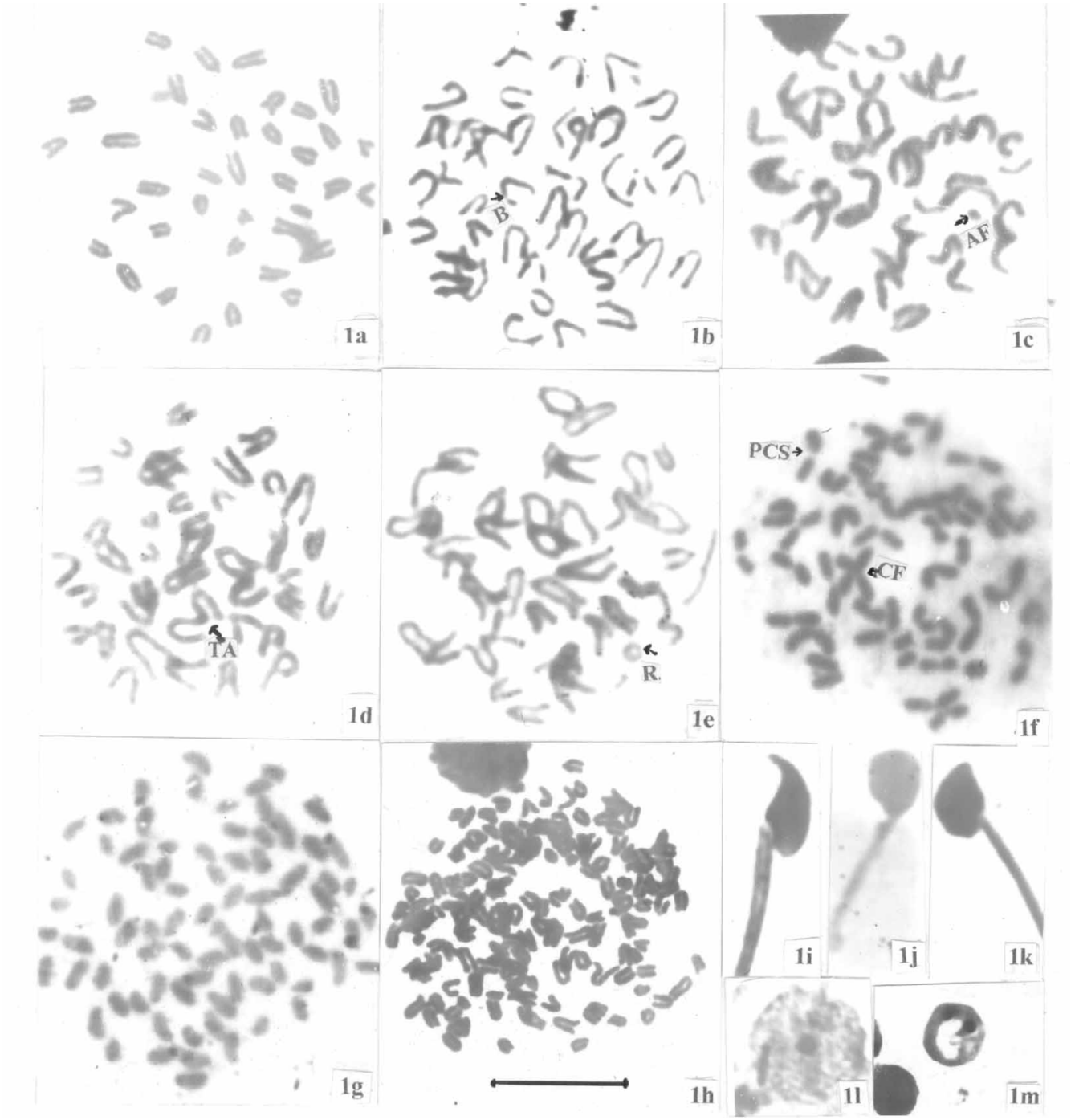

Figure 1
a-m: Photomicrographs of metaphase complements showing normal set of chromosomes (Ia), and chromosome aberrations like break (B, 1b), acentric fragment (AF, 1c), terminal association (TA, 1d), ring (R, 1e), centric fusion and precocious centromeric separation (If), C mitosis (1g) polyploidy (1h); sperm with normal (1i), and abnormal (1j-1k) head morphology; normo- (1l), and poly- (1m) chromatic erythrocytes. Bar represents 10 μm.

Cytogenetical Studies

As compared to normal metaphase plates (Fig-1a) which did not normally reveal any aberrations, various types of chromosome aberrations of both major (Figs. 1b,1c,1d,1e,1h) and minor nature (Fig. 1f,1g) were encountered in certain metaphase plates of mice that received p-DAB and/or PB treatments (see table 2). The total frequencies of aberrations were found to be maximum in the p-DAB and PB fed mice and the aberrations were considerably reduced in both the drug fed series (Fig. 2). However while Ch-30 appeared to protect the bone marrow cells at a higher scale at 60 and 90 days, Ch-200 showed greater protection at 120 days ($p < 0.001$; see table 2). The mice fed PB alone had less number of chromosome aberrations than in the p-DAB + PB treated series and differences in the % of the CA, when compared with that of the normal controls were found to be statistically significant at 90 ($p < 0.05$) and 120 days ($p < 0.01$).

Micronucleated erythrocytes

Data on occurrence of micronuclei in polychromatic (Fig. 1m) erythrocytes (PCE) and normochromatic (Fig. 1l) erythrocytes (NCE) have been provided in table 2. The percentages of MN were highest in the p-DAB and PB fed mice. Both Ch-30 and 200 feeding reduced the occurrence of MN. Ch-200 showed more pronounced action ($p < 0.05$) at 60 and 120 days (Fig. 3). PB itself produced a few micronucleated erythrocytes not significantly different from that of normal controls (table-2).

Mitotic index

In both Ch-30 and 200 fed mice, the MI was much less than in the p-DAB plus PB fed mice and the protection was statistically significant ($p < 0.05$ through $p < 0.001$). The mitotic index in the PB fed mice was only slightly more than in the normal control series (table-2, Fig. 4).

Sperm head anomaly

As against sperm with normal head morphology (Fig. 1i), quite high incidence of sperm showing some form of abnormal head morphology (Fig. 1j,1k) has been recorded in the different treatment series (see table 2, Fig. 5). Both Ch-30 and Ch-200 reduced considerably the percentages of sperm with abnormal head morphology and the differences were statistically significant ($p < 0.05$ through $p < 0.001$). The feeding of PB alone also produced abnormal sperm in greater number than in the normal control and the differences were statistically significant at 60 days ($p < 0.05$) and at 120 days ($p < 0.01$).

Lipid peroxidase activity

The lipid peroxidase activity was the highest in all the three tissues at all fixation intervals in the p-DAB+ PB treated series except at 90 and 120 days in the liver where the activity was the highest in the p-DAB + PB+Ch-200 fed mice (Fig. 6). However, interestingly enough, while the lipid peroxidase enzyme activity was generally much reduced in the Ch-200 fed mice as compared to Ch-30 fed mice in spleen and kidney, the lower micro dose i.e. Ch-30 appeared to reduce the activity in liver ($p < 0.05$) more than that of Ch-200 at all fixation intervals ($p < 0.01$ to $p < 0.001$, see table 3). The feeding of PB alone produced similar or marginally increased activity in spleen, kidney and liver at 60 and 90 days but the activity became appreciably higher in the liver and kidney at 120 days but not in the spleen.

Alkaline phosphatase activity

The alkaline phosphatase activity (AlkPA) in the p-DAB + PB fed mice was highest in spleen and liver at all the three fixation intervals while the activity declined in both Ch-30 and Ch-200 fed mice ($p < 0.05$, $p < 0.001$, see table-3, Fig. 7). Ch-200 showed greater efficacy in reducing AlkPA than that of Ch-30 fed mice except at 90 days in case of spleen. In the kidney, however, although p-DAB + PB showed, except for a few cases, a high degree of AlkPA at all fixation intervals, the activity could not be reduced by Ch-200 till at 120 day ($p < 0.05$) while the activity level became actually higher at 60 and 90 days. However, Ch-30 could reduce the activity to some extent at all the fixation intervals although the differences were not statistically significant.

Acid phosphatase activity

The acid phosphatase activity (AcPA) in spleen, liver and kidney was very high in p-DAB + PB fed mice and the activity was slightly declined in the Ch-30 and Ch-200 fed mice except at some fixation intervals where actually it was higher than in the p-DAB + PB fed mice. The differences were not statistically significant in most cases and only some favorable modulation was noted in liver and kidney at 120 days ($p < 0.05$, $p < 0.001$; see table 3, Fig. 8).

Discussion

It had earlier been conclusively demonstrated that feeding of carcinogenic azo dyes produced liver damage followed by regeneration of parenchymal cells, proliferation of bile ducts and connective tissue, and at later stages tumors developed from liver parenchyma that ended up with neoplastic characteristics [10]. Further, it was also demonstrated that dietary PB had positive carcinogenic effect only when fed with the azodye 2-methyldiaminoazobenzene, but neither of these two when fed alone showed positive hepatocarcinogenesis in both mice and rat [11,12]. It is generally accepted that covalent binding of the metabolites of p-DAB (e.g. MAB, AAB etc.) with DNA is a major carcinogenic factor [13]. In the present study, the only PB fed mice also did not develop tumors in liver, while those fed with p-DAB + PB developed tumors. However, interestingly enough, every 2 out of 5

Table 2: Showing frequency distribution of mitotic indices (MI) in 5000 cells, chromosome aberration (CA) in 500 cells, micronuclei (MN) in polychromatic (PCE) and normochromatic (NCE) erythrocytes in 5000 cells and sperm head anomaly (SHA) in 5000 sperm in mice treated with p-Dimethylaminoazobenzene (DAB) + Phenobarbital (PB), DAB + PB + Chelidonium (Ch)-30 and DAB + PB + Chelidonium (Ch)-200 and their Phenobarbital treated and normal controls. Equal number of cells/sperms observed from each of 5 mice of a set.

		Mitotic Index		Chromosome Aberration				Micronucleated Erythrocytes					Sperm Head Anomaly	
Fixation intervals (days)	Series	% of MI (% ± SE)	% of Prot.	% of Major CA	% of Other CA	Total CA (% ± SE)	% of Prot.	% of MN in PCE	% of MN in NCE	P/N	Total MN (% ± SE)	% of Prot.	Total SHA (% ± SE)	% of Prot.
	Normal	1.32 ± 0.08		1.4	1.4	2.80 ± 0.58		0.04	0.26	0.89	0.16 ± 0.05		0.44 ± 0.12	
	PB	2.10 ± 0.48		1.6	1.8	3.40 ± 0.98		0.14	0.28	0.74	0.16 ± 0.05		1.08 ± 0.24	
60	DAB + PB	8.70 ± 0.79		5.4	10.4	15.8 ± 2.11		0.53	0.88	0.84	0.72 ± 0.14		2.62 ± 0.28	
	DAB + PB + Ch-30	4.66 ± 0.34	4.04[b]	4.4	4.6	9.0 ± 0.78	6.8[a]	0.41	0.61	0.79	0.52 ± 0.09	0.20	1.66 ± 0.30	0.96[a]
	DAB + PB + Ch-200	3.36 ± 0.37	5.34[c]	4.6	8.2	12.8 ± 3.87	3.0	0.45	0.26	0.497	0.32 ± 0.08	0.42[a]	0.8 ± 0.11	1.82[c]
	Normal	1.32 ± 0.08		1.4	1.4	2.80 ± 0.58		0.04	0.26	0.89	0.16 ± 0.05		0.44 ± 0.12	
	PB	1.58 ± 0.14		2.2	3.0	5.20 ± 0.86		0.13	0.22	0.83	0.180.08		0.52 ± 0.09	
90	DAB + PB	6.40 ± 1.14		6.6	4.2	10.8 ± 1.20		1.17	0.27	0.80	0.67 ± 0.10		2.90 ± 0.27	
	DAB + PB + Ch-30	3.08 ± 0.29	3.3[a]	4.4	4.8	9.20 ± 1.86	1.6	0.24	0.55	1.33	0.37 ± 0.10	0.16	2.20 ± 0.26	0.70
	DAB + PB + Ch-200	2.90 ± 0.34	3.5[a]	4.6	5.6	10.2 ± 1.56	0.6	0.29	0.48	1.16	0.38 ± 0.11	0.16	1.66 ± 0.16	1.24[b]
	Normal	1.32 ± 0.08		1.4	1.4	2.80 ± 0.58		0.04	0.26	0.89	0.16 ± 0.05		0.44 ± 0.12	
	PB	2.12 ± 0.30		2.4	4.2	6.60 ± 0.93		0.21	0.11	0.91	0.16 ± 0.08		1.12 ± 0.12	
120	DAB + PB	8.20 ± 0.26		8.6	13.0	22.4 ± 0.25		0.63	0.54	0.80	0.58 ± 0.15		1.58 ± 0.31	
	DAB + PB + Ch-30	8.72 ± 0.65	-0.52	7.8	7	14.8 ± 2.18	7.6[b]	0.49	0.27	0.34	0.32 ± 0.08	0.32	0.40 ± 0.12	1.18[b]
	DAB + PB + Ch-200	6.44 ± 0.35	1.8[b]	3.8	4.2	8.0 ± 0.32	14.4[c]	0.12	0.19	2.18	0.14 ± 0.06	0.43[a]	0.38 ± 0.07	1.20[b]

Major Types of CA include: Break; Centric fusion; Translocation; Fragment; Pulverisation; Ring; Terminal association; Polyploidy.;Aneuploidy. **Other types of CA include:** Erosion, Constriction, Precocious centromeric separation, Centromericstrecthing, Stickiness, C-mitotic effect. SE= standard error, a= $p < 0.05$, b= $p < 0.01$, c= $p < 0.001$. Prot.= Protection given by the drug

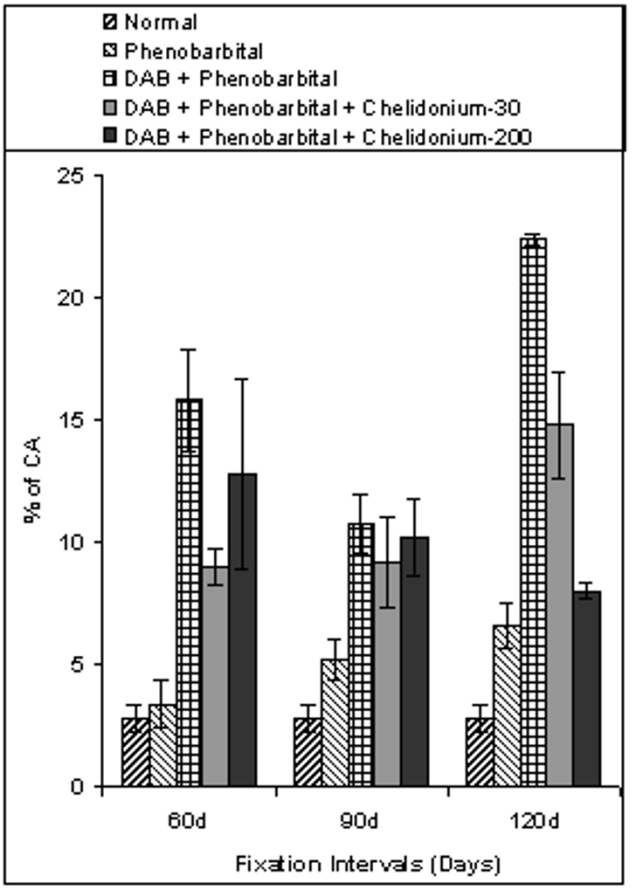

Figure 2
Showing % of CA in different series of mice at different fixation intervals.

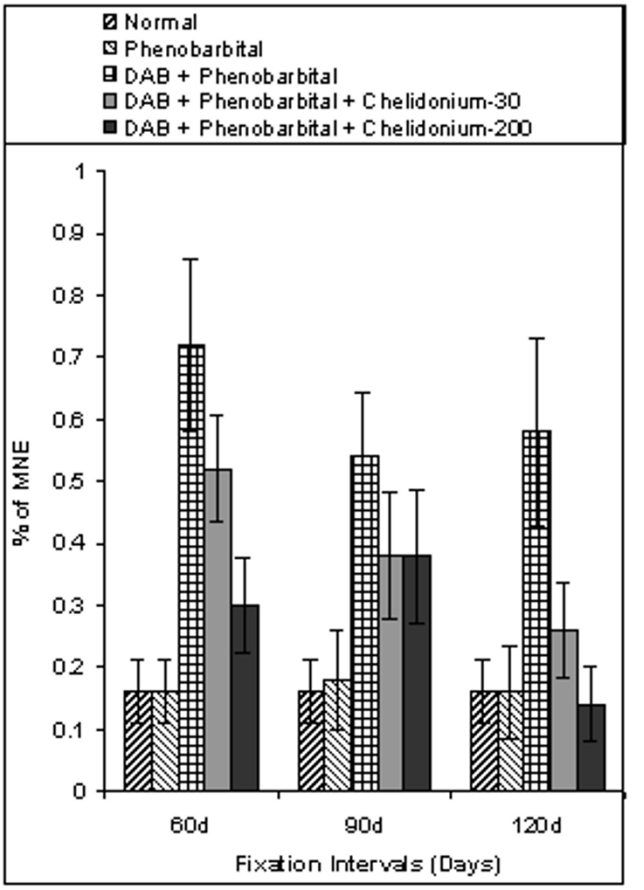

Figure 3
Showing % of MNE in different series of mice at different fixation intervals.

mice, that is, about 40% of mice that received both p-DAB and PB along with either Ch-30 or Ch-200 did not develop tumors in liver while all mice fed p-DAB plus PB, but no Chelidonium developed tumors. This seems to be a dramatic finding as neither of these potentized drugs Ch-30 or Ch-200 had literally a single molecule of original drug substance in their diluted forms and were yet capable of reducing/delaying tumor growth in mice. Such agents that can antagonise or render protection at various levels of carcinogenesis are always considered very important, particularly so when they can be administered in micro doses and they do not have any ill-effects/side-effects of their own. When microdoses of either of these drugs were fed alone to healthy mice in similar doses, and dissected at corresponding fixation intervals, no tumor was found in their liver, nor was any genotoxic effect found from the assay of their chromosomes, sperm heads, or micronuclei. Earlier, Roberfroid *et al*[14] reported that micro doses of PB 9C positively reduced the incidence of tumors and mortality in rats chronically fed with another carcinogen Acetylamino fluorine along with PB. However, these authors did not consider any of the protocols used in the present study. Fisher [15] reported that the DNA repair mechanisms of cultured mammalian cells *in vitro* could be stimulated by very small doses of mutagens while working with human lymphocytes.

Extensive toxicological investigations have now established that increase in lipid peroxidation, alkaline and acid phosphatase activities along with decreased level of glutathione actually denote cytotoxicity and hepatocellular dysfunction [16–19]. The favorable modulations of some of these enzymes, chromosomal and sperm head damages noted in the Chelidonium fed mice as compared to drug unfed p-DAB plus PB fed mice were very significant. Thus it was suggestive that the micro doses of Ch i.e. 30 and 200 had positive protective effects against tumors induced by p-DAB plus PB, and that Ch-200 appeared to have marginally better effects at the longer intervals. Incidentally, the efficacy of serial agitated dilutions of home-

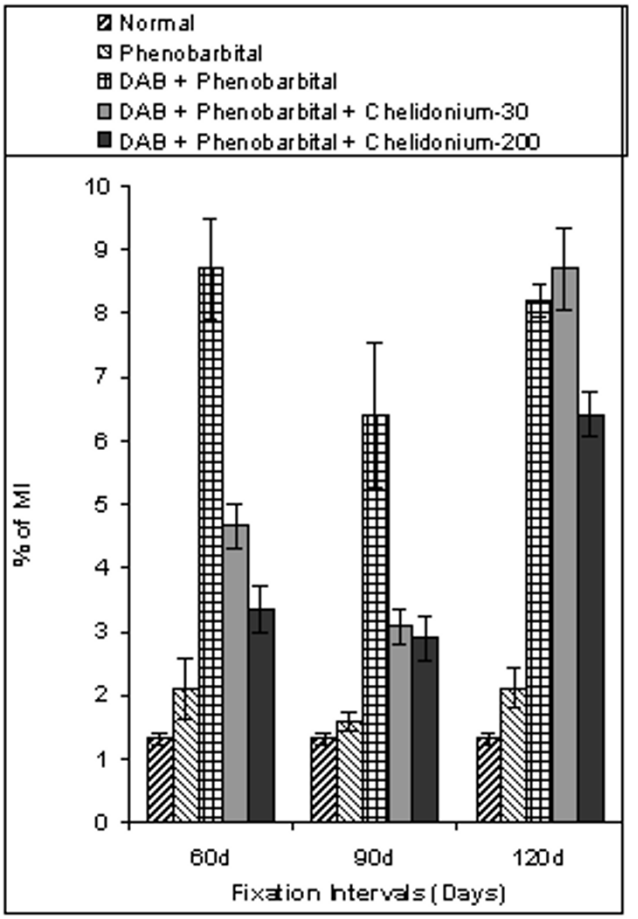

Figure 4
Showing % of MI in different series of mice at different fixation intervals.

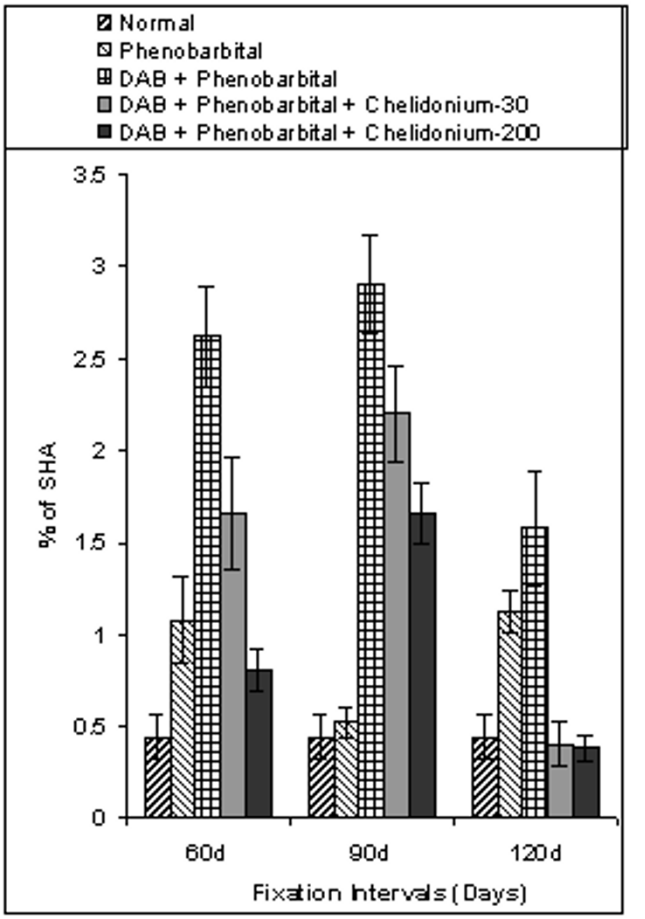

Figure 5
Showing % of SHA in different series of mice at different fixation intervals.

opathic drugs in experimental toxicology had also been convincingly advocated by meta-analysis done by Linde *et al*[20].

p-DAB and its metabolites have been reported to cause oxidative DNA damage [13], which could also be attributable to the various types of chromosome aberrations encountered in the present investigation. The formation of adducts, DNA-copper-hydroperoxo complexes, etc as suggested by Ohnishi *et al*[13], could also play an important role in the carcinogenic processes of p-DAB. Therefore antagonism in an unknown manner to either formation of various metabolites of p-DAB or else in their formation of adducts in DNA could have been one of the major ways by which the potentized Chelidonium acted in the mice treated with the azo dye to reduce the chromosomal damage.

It is difficult to understand precisely at the present state of our knowledge how the ultra low doses of Chelidonium could achieve such spectacular protective changes which were amply demonstrable in the present study with the different protocols used. One hypothesis to explain the possible mechanism of action of the micro doses could be as follows. Since these low doses of the medicines were administered orally, their actions could possibly be mediated through the receptor systems located on tongue and the oral cavity. The drugs must have emanated specific signals in the receptor cells that could activate specific region of brain (presumably hypothalamus) in a manner that could possibly help elicit further signals to activate or repress certain transcriptional activities of specific regions of DNA meant for restoration of the damages caused due to the carcinogenic interaction. One way to test this hypothesis can be either to measure the activity of the signal transduction system through estimation of secondary messenger (i.e cyclic AMP) or else to block the pathway se-

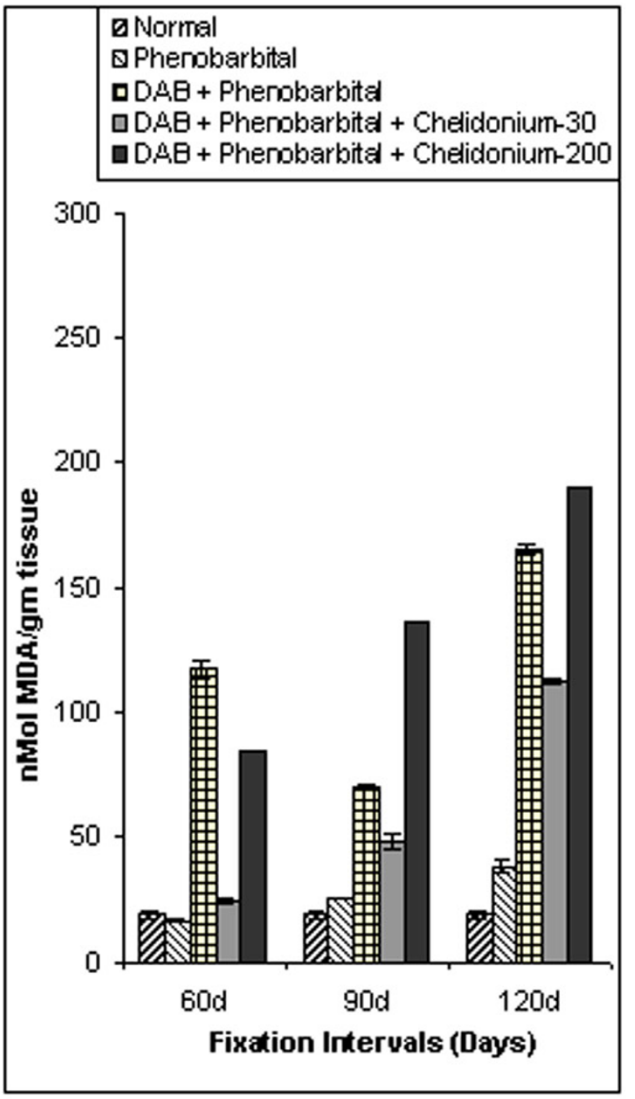

Figure 6
Showing lipid peroxidase activity in liver of experimental mice at different fixation intervals.

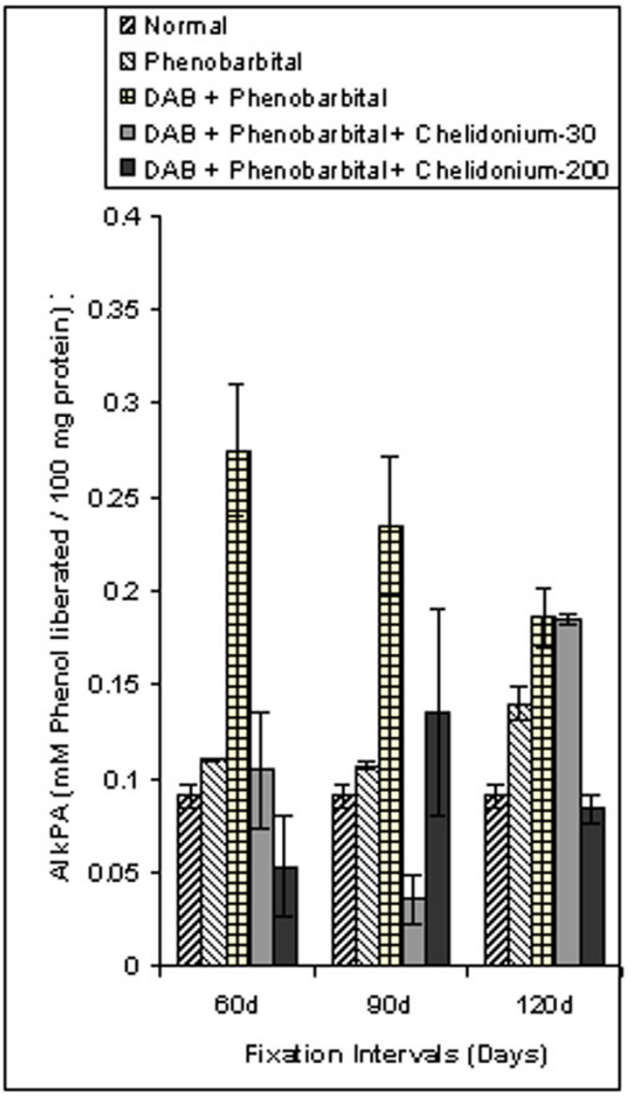

Figure 7
Showing alkaline phosphatase activity in liver of experimental mice at different fixation intervals.

lectively after use of any drug in such microdoses. Incidentally, when some potentized homeopathic drugs were used along with Actinomycin D, a transcription blocker, the homeopathic drugs failed to elicit the desired protective levels against chromosomal and other cytogenetical damages produced by arsenic intoxication and by ultrasonic sound waves [21,22] as compared to when the drugs were administered alone (i.e. without Actinomycin-D). Thus the repair mechanisms were essentially mediated through active gene action. Khuda Bukhsh [23] proposed a hypothesis to explain the possible mechanism of action of such micro doses based on many circumstantial evidences [21–33] that the micro doses act through the regulation of relevant gene expression by eliciting impulses similar to that of some hormones and enzymes. That can suitably explain the mechanisms involved in the repair of damaged chromosomes or sperm head by the application of the micro doses. This can also explain the antagonizing action against tumorigenesis/carcinogenesis which is essentially a multi-gene and multi-step process in majority of cancer including hepatocarcinoma [34]. Since the activation of proto-oncogene to oncogene is the key event for the transformation of the normal hepatocyte to a malignant liver tumor cell and that this process is controlled by the interactions of many tumorigenic and tumor supressor genes (e.g. p53 gene), it may be speculated that the mi-

Table 3: Mean activities of lipid peroxidase (nMol MDA/gm wet tissue), acid and alkaline phosphatase activities (in terms of mM Phenol liberated/100 mg protein after 30 min of incubation at 37°C), in different organs of mice treated with p Dimethylaminoazobenzene (DAB) + Phenobarbital (PB), DAB + PB + Chelidonium (Ch)-30 and DAB + PB + Chelidonium (Ch)-200 against their normal and phenobarbital control.

		Lipid peroxidase activity						Alkaline phosphatase activity						Acid phosphatase activity					
Fixation Interval (Days)	Series	Liver		Kidney		Spleen		Liver		Kidney		Spleen		Liver		Kidney		Spleen	
		Activity ± SE	% Prot.	Activity ± SE	% Prot.	Activity ± SE	% Prot.	Activity ± SE	% Prot.	Activity ± SE	% Prot.	Activity ± SE	% Prot.	Activity ± SE	% Prot.	Activity ± SE	% Prot.	Activity ± SE	% Prot.
	Normal	18.72 ± 1.27		16.67 ± 0.26		25.44 ± 0.68		0.09 ± 0.01		0.12 ± 0.03		0.09 ± 0.01		0.10 ± 0.002		0.09 ± 0.004		0.10 ± 0.003	
	PB	16.58 ± 0.43		15.85 ± 1.39		17.35 ± 0.45		0.11 ± 0.001		0.11 ± 0.004		0.11 ± 0.002		0.18 ± 0.02		0.15 ± 0.02		0.15 ± 0.02	
60	DAB +PB	117.48 ± 3.80		134.41 ± 1.08		207.95 ± 3.01		0.28 ± 0.04		0.25 ± 0.02		0.15 ± 0.01		0.30 ± 0.03		0.29 ± 0.05		0.27 ± 0.04	
	DAB+PB+Ch30	24.26 ± 0.78	93.22[a]	29.04 ± 1.05	105.37[c]	44.32 ± 1.14	163.63[b]	0.11 ± 0.03	0.17	0.22 ± 0.01	0.03	0.07 ± 0.03	0.07[a]	0.30 ± 0.03	0	0.30 ± 0.05	0.01	0.26 ± 0.03	0.01
	DAB+PB+Ch200	84.67 ± 0.27	32.81	19.737 ± 0.45	114.67	48.71 ± 3.28	159.24[b]	0.05 ± 0.03	0.22	0.28 ± 0.04		0.04 ± 0.01	0.11	0.24 ± 0.03	0.06	0.22 ± 0.04	0.07	0.30 ± 0.03	0.03
	Normal	18.72 ± 1.27		15.85 ± 1.39		25.44 ± 0.68		0.09 ± 0.01		0.12 ± 0.03		0.09 ± 0. 01		0.10 ± 0.002		0.09 ± 0.004		0.10 ± 0.003	
	PB	25.21 ± 0.31		16.58 ± 0.62		21.88 ± 0.98		0.11 ± 0.002		0.12 ± 0.003		0.11± 0. 003		0.19 ± 0.003		0.18 ± 0.01		0.18 ± 0.003	
90	DAB +PB	69.25 ± 0.75		61.42 ± 0.61		91.99 ± 5.20		0.24 ± 0.04		0.27 ± 0.04		0.17± 0. 01		0.36 ± 0.04		0.30 ± 0.01		0.24 ± 0.02	
	DAB+PB+Ch30	48.77 ± 3.07	20.48	22.12 ± 0.76	39.30[c]	30.95 ± 3.36	61.04[c]	0.04 ± 0.01	0.19	0.26 ± 0.05	0.01	0.06 ± 0. 04	0.11[a]	0.32 ± 0.07	0.04	0.29 ± 0.04	0.01	0.28 ± 0.04	0.04
	DAB+PB+Ch200	135.74 ± 0.22	-66.49	22.18 ± 0.64	39.24[b]	30.49 ± 0.75	61.51[c]	0.14 ± 0.06	0.09	0.34 ± 0.02		0.09 ± 0. 04	0.08	0.30 ± 0.04	0.06	0.29 ± 0.02	0.01	0.30 ± 0.04	0.06
	Normal	18.72 ± 1.27		15.85 ± 1.39		25.435 ± 0.68		0.09 ± 0.01		0.12 ± 0.03		0.09 ± 0. 01		0.10 ± 0.002		0.09 ± 0.004		0.10 ± 0.003	
	PB	38.63 ± 2.79		44.96 ± 1.49		28.72 ± 0.65		0.14 ± 0.003		0.12 ± 0.01		0.17 ± 0. 002		0.20 ± 0.001		0.15 ± 0.01		0.18 ± 0.001	
120	DAB+PB	165.43 ± 1.92		138.65 ± 2.09		274.55 ± 1.08		0.19 ± 0.02		0.31 ± 0.02		0.15 ± 0. 02		0.40 ± 0.07		0.34 ± 0.05		0.29 ± 0.03	
	DAB+PB+Ch30	113.04 ± 1.14	52.39[a]	89.79 ± 0.48	48.86	92.56 ± 0.18	181.99[c]	0.19 ± 0.003	0.001	0.27 ± 0.05	0.04	0.15 ± 0. 003	0.01	0.50 ± 0.05	0.1	0.67 ± 0.09	0.33	0.22 ± 0.05	0.06
	DAB+PB+Ch200	190.25 ± 0.48	-25.82	18.21 ± 0.48	120.45[b]	110.51 ± 0.36	160.04[c]	0.08 ± 0.01	0.102	0.15 ± 0.06	0.16[a]	0.10 ± 0. 01	0.05[a]	0.28 ± 0.02	0.12[a]	0.16 ± 0.02	0.17	0.24 ± 0.01	0.05

SE= Standard error, a =p < 0.05, b= p < 0.01, c= p < 0.001. Prot. = Protection given by the drug.

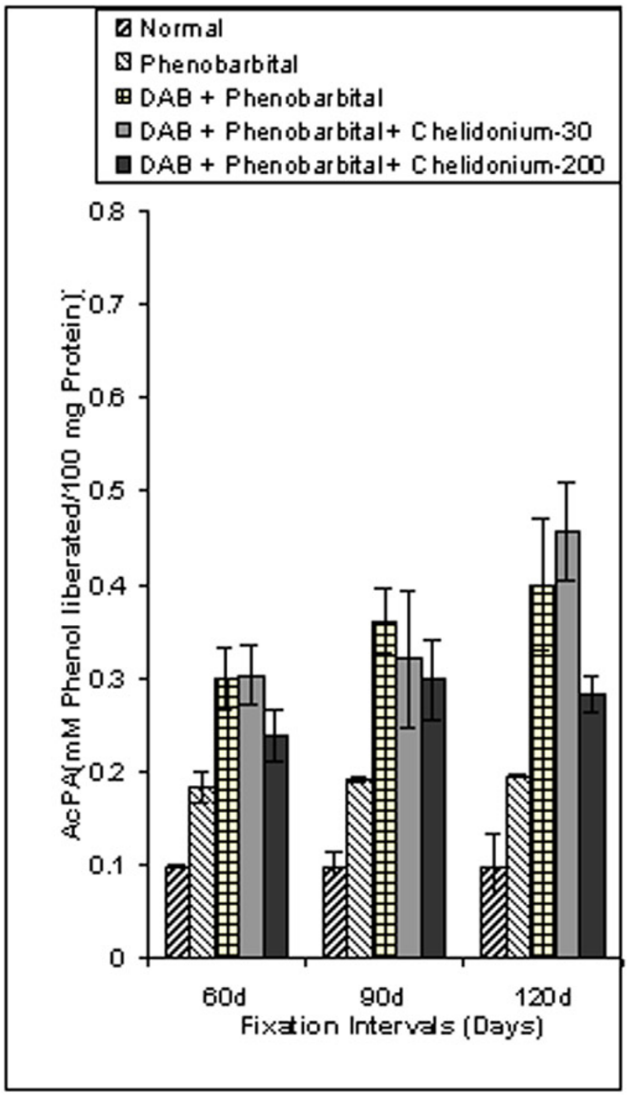

Figure 8
Showing acid phosphatase activity in liver of experimental mice at different fixation intervals.

cro doses of the drug might have interfered with the process of carcinogenesis either by actively modifying action of certain of these genes responsible for the transformation of cells to cancerous ones by release of specific transcription factors, or else by activating certain tumor-suppressor genes in an unknown manner. A careful study of telomerase activity in these experimental mice can also be helpful in understanding the underlying genetic mechanism. In fact the modulating effects of the drug on restoration of damage caused to several gene regulated phenomena like enzyme activities, chromosome and sperm structure etc would further strengthen the contention that these drugs possibly acted through regulatory actions on a number of key genes, related not only to the structure and normal functioning of liver hepatocytes, but also to the ones meant for maintaining integrity of bone marrow chromosomes and sperm head.

Conclusion

From the present investigation, it becomes evident that ultra-low doses of the homeopathic drug Chelidonium are also capable of rendering anti-tumor and anti-genotoxic activities against azo-dye induced hepatocarcinoma in mice. This would encourage their use even in cancerous liver disorders with greater degree of assurance of their efficacy. Further, it would hopefully open up new vistas for understanding the little known mechanism of action of the homeopathic microdoses which in many instances do not even contain a single molecule of the original drug substance.

Acknowledgements

Part of this work was financially supported by the University of Kalyani. Grateful acknowledgements are made to Prof. G.K. Manna, Professor Emeritus, Prof. S.P. Sen and Dr S.P. De for their encouragements and appreciation of the work and to Indian Institute of Chemical Biology, Kolkata for their help in collecting certain references.

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Pre-publication history

The pre-publication history for this paper can be accessed here:

http://www.biomedcentral.com/1472-6882/2/4/prepub