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Evaluation of protective potentials of a potentized homeopathic drug, *Chelidonium majus*, during azo dye induced hepatocarcinogenesis in mice

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Several cytogenetical and enzymatic protocols were used to test if two microdoses of *Chelidonium majus*, namely Chelidonium-30 (Ch-30) and Chelidonium-200 (Ch-200), used as homeopathic drugs, showed anti-tumor activity and also favorably modulated genotoxic damages produced by an azo dye in mice at several intervals of fixation. Different sets of healthy mice were fed: (i) hepatocarcinogen, p-dimethylaminoazobenzene (p-DAB, initiator) + phenobarbital (PB, promoter), (ii) only p-DAB, (iii) only PB, and (iv) neither p-DAB nor PB (normal control). Mice fed with p-DAB + PB were divided into different sets that were also fed either Ch-30 (v) or Ch-200 (vi) or diluted alcohol (vii), the "vehicle" of the microdoses of Chelidonium. All mice of group (i), a few of group (ii) and group (vii) and none of groups (iii) and (iv) developed tumors in liver at the longer intervals of fixation. The frequencies of chromosome aberrations (CA), micronucleated erythrocytes (MN), mitotic index (MI) and sperm head abnormality (SHA) were much higher in groups (i) and (vii) mice than in groups (ii), (iii) and (iv) mice at all fixation intervals. However, in mice of both groups (v) and (vi), the frequencies of CA, MN, SHA were strikingly less than those of groups (i) and (vii), and moderately less than those of groups (ii) and (iii). Both Ch-30 and Ch-200 also modulated favourably some toxicity marker enzymes like acid and alkaline phosphatases, peroxidases, glutamate oxalo-acetate and glutamate pyruvate transaminases in liver, kidney and spleen tissues of the carcinogen fed mice. The microdoses of Chelidonium having no visible ill effects of their own, may be strong candidates for use in delaying/protecting liver cancer.

Keywords: p-DAB, Hepatocarcinogenesis, Microdoses, Chelidonium, Genotoxicity, Toxicity marker enzymes.
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Chelidonium majus L. (Papaveraceae), commonly known as Greater Celandine, is a plant of great interest for its use in various diseases as herbal medicines in both European and Asian countries. Crude extracts of various parts like root, shoot and leaves have been reported to have several isoquinoline alkaloids, such as, sanguinarine, chelidonine, chelerythrine, berberine and coptisisine etc. 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 homeopathic mode of treatment, various microdoses of Chelidonium herb extract are routinely used against several forms of liver ailments with some specific disease symptoms, including that of liver cancer[6]. But, to our knowledge, whether microdoses of *Chelidonium majus* (Ch) could also have similar anti-tumor or anti-clastogenic activities had not been experimentally studied so far in mice *in vivo*. Therefore the present investigation has been undertaken primarily with the objectives: to examine if two microdoses of Chelidonium (Ch-30 and 200), prepared as per homeopathic procedure could show (i) anti-tumor 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 toxicity marker enzymes like acid and alkaline phosphatases, peroxidase, glutamate oxalo-acetate and glutamate pyruvate transaminases in various tissues during azo dye induced hepatocarcinogenesis in mice.

Materials and Methods

Inbred strain 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), University of Kalyani,

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were used. Mice were provided food and water *ad libitum*. The food was made up of wheat, gram and powdered milk without any animal protein supplement. A group of 70 healthy mice weighing between 25-30 g were used for each of the three long term fixation intervals viz. 60, 90 and 120 days and a group of 30 mice were taken for the shorter fixation periods (7 days, 15 days and 30 days) (Table 1). Each group of 70 mice (for longer intervals) were divided into seven different sets consisting of 10 mice each for normal, phenobarbital (PB), p-Dimethylamino-azobenzene (p-DAB), p-DAB + PB, p-DAB + PB + alcohol, p-DAB + PB + Ch-30 and p-DAB + PB + Ch-200 series. The first set of mice was allowed normal low protein diet (group i, normal negative control). The second set of mice was fed low protein diet and only 0.05% aqueous solution of PB instead of pure water (group ii). The third set of mice was provided with low protein diet mixed with 0.06% p-DAB (Sigma, D-6760), and water *ad libitum* (group iii). The fourth set of mice was fed low protein diet mixed with 0.06% p-DAB and water *ad libitum* till 30 days after which the water was replaced with 0.05% aqueous solution of PB till they were sacrificed (group iv). The fifth set of mice was fed as in group iv, but along with also fed dilute succussed alcohol (group v, the "vehicle" of Ch-30 and Ch-200 being ethyl alcohol; 0.06 ml of 90% ethyl alcohol diluted with 20 ml of distilled water and feeding 0.06 ml of this dilute alcohol at the corresponding intervals as that of either the Ch-30 or Ch-200, to which it served as positive control). The sixth set of mice (group vi) was fed as in group iv, but also along with Ch-30, for only longer fixation intervals (60, 90 and 120 days, when the tumors could be visible on livers of all p-DAB plus PB fed mice (see Table 1) The seventh set of mice was fed as in group iv, but also along with Ch-200 for all fixation intervals (group vii). Ch-30 was fed thrice a day (0600, 1200 and 1800 hrs) from first day onward of p-DAB feeding for 7 days, and then twice a day (0600 and 1800 hrs) till they were sacrificed (at longer fixation periods of 60, 90 and 120 days). Ch-200 was fed twice a day (0600 and 1800 hrs) all along till they were sacrificed (at all fixation periods starting from 7 days through 120 days). For the shorter fixation intervals (7,15 and 30 days), PB was fed to mice along with p-DAB from day 1 onwards in the p-DAB plus PB fed series. A separate group of 12 healthy mice each were only fed either Ch-30 or Ch-200 at the corresponding time when the drug was fed to the p-DAB plus PB treated mice and 2 of them sacrificed at each of the six different fixation intervals for checking induction of liver tumor, if any, or any change in the genotoxic parameters studied. But since no significant differences in values between the only Ch-30 or Ch-200 fed group and the normal healthy mice fed distilled water (negative control group) could be observed, and neither any sign of tumor development observed, the last mentioned only drug-fed mice series were no more extended for reasons of economy on mice lives and the data thereof not separately mentioned to keep the tables within manageable limits.

Preparation of the microdoses of the drug—The two microdoses of Chelidonium were procured from "HAPCO", 165, Bipin Behari Ganguli Street, Kolkata. The microdoses were prepared as per the standard procedure. The dry drug material of *Chelidonium majus* (whole plant) was extracted in 44% ethyl alcohol (i.e. the "mother tincture"). The mother tincture (1 ml) was subsequently diluted with 99ml 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 and dilute alcohol—Ch-30 and Ch-200 (1 ml each) were finally diluted separately with 20 ml of double distilled water to make the stock solution of Ch-30 and Ch-200, respectively. Similarly, 1 ml of 90% ethyl alcohol was diluted with 20 ml of distilled water to make the stock solution of alcohol.

Feeding procedure and dose—Each mouse was fed 1 drop (0.06 ml) of either Ch-30, Ch-200 or alcohol from the stock solutions at a time with the aid of a fine pipette as per requirement of a particular series.

Laboratory Methodology

Cytogenetic assay—The standard and widely practiced cytogenetic protocols like assays of chromosome aberrations, micronuclei, mitotic index and sperm head anomaly have been adopted in the present study.

Mice were injected (ip) with 0.03% colchicine @ 1ml/100 gm body weight 90 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[7] followed by Giemsa staining for scoring bone marrow chromosome aberrations. Chromosome aberrations of

various nature have been pooled into two categories: the "major" type comprising aberrations like break, fragments, ring etc. and the "other" types comprising less significant aberrations like gaps, erosions, etc. 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 [8]. 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 nondividing and dividing cells were recorded and their ratios ascertained[8].

For sperm head anomaly (SHA), the technique of Wyrobek[9] was adopted with minor modifications. 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 min 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* [10]. 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 min the extinction was read at 750 nm against appropriate blank in spectrophotometer (Shimadzu, Double beam Spectrophotometer UV-180, Japan).

The lipid peroxidation activity was estimated from the supernatant by the method of Buege and Aust [11]. Sample (1 ml; homogenate containing 0.1-0.2 mg protein) was mixed thoroughly with 2 ml of TCA-TBA-HCl (15% w/v TCA and 0.375% w/v TBA in 0.25N HCl). The absorbance of the sample was determined at 535nm 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 $M^{-1}cm^{-1}$.

For the study of acid and alkaline phosphatases method of Walter and Schutt [12] 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 min. Then 2 ml of 0.1 *N* NaOH was added. The absorbance was measured at 405 nm against the standard.

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 ^{0}C for 30 min, then 10 ml of 0.05N NaOH was added and the absorbance was measured at 405 nm against a blank.

For estimation of glutamate oxalo-acetate transaminase (GOT) and glutamate pyruvate transaminase (GPT) activities, the method of Bergmeyer and Brent [13] was followed with some minor modifications. For GOT, 0.1ml of tissue homogenate was made to react with 0.5 ml of the substrate solution L-asparate and was incubated for 60 min at 37°C. This was followed by addition of 0.5 ml of dinitrophenylhydrazine (DNPH) and then by 5 ml 0.4*N* NaOH. The absorbance was measured at 510 nm.

For the analysis of GPT, 0.1 ml of tissue homogenate was made to react with 0.5 ml of the substrate solution (L-alanine) and incubated for 30 min at 37°C. Rest of the procedure was same as that of GOT and the absorbance was also measured at 510nm.

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 uniformity was maintained in scoring data of both treated and control sets of mice.

Results

Tumor growth—Out of the total of 210 mice fed either p-DAB, or p-DAB plus PB, with or without feeding them the homeopathic drug, or the "vehicle", and sacrificed at longer fixation intervals, livers in some 91 mice showed distinct sign of tumor formation in the form of pale reddish multiple nodules (Table 1). While all mice fed only p-DAB plus PB developed tumorous nodules in liver and had appreciably

enlarged spleen, a total 39 out of 75 mice that received either Ch-30 or Ch-200 alongside p-DAB plus PB did not show sign of tumor. Out of 45 mice fed only p-DAB, 20 also showed signs of tumor development at longer fixation periods.

Cytogenetical studies—As compared to normal metaphase plates (Fig.1) which did not normally reveal any aberrations, various types of chromosome aberrations of both major (Figs. 2-6) and minor nature were encountered in certain metaphase plates of mice that received p-DAB and/or PB treatments (Tables 2 and 3). The total frequencies of aberrations were found to be maximum in p-DAB + PB fed mice and the aberrations were considerably reduced in both the drug fed series. 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, (Table-3). The ameliorating effects of Ch-200 were also quite pronounced in the earlier fixation periods like 7, 15 and 30 days though amelioration in cytogenetical effects was not so pronounced at these intervals. The mice fed either PB or p-DAB alone had less number of chromosome aberrations than in the p-DAB + PB treated series, but had more aberrations when compared to normal controls, the differences being statistically significant at all fixation intervals (Tables 2 and 3).

Micronucleated erythrocytes—Data on occurrence of micronuclei in normochromatic (NCE) (Figs. 7 and 8) erythrocytes and polychromatic (Fig. 9) erythrocytes (PCE) have been given in Tables 2 and 3. The percentages of MN were highest in p-DAB + PB fed mice at all the fixation intervals, while the treatment with either p-DAB or PB also induced quite a few micronuclei. Both Ch-30 and 200 feeding reduced the occurrence of MN. Ch-200 showed more pronounced action at 7, 60 and 120 days. PB itself produced a few micronucleated erythrocytes not significantly different from that of normal controls.

Mitotic index—In both Ch-30 and 200 fed mice, the MI was much less than that in p-DAB + PB fed mice and the protection was statistically significant for Ch-200 at 7, 60, 90 and 120 days, and for Ch-30 at 60 and 90 days. The mitotic index in the p-DAB fed mice was also appreciably higher than in the normal control series, while in the only PB fed mice, the MI was only marginally higher than in the normal control (Tables 2 and 3).

Sperm head anomaly—As against sperm with normal head morphology (Fig.10), quite high incidence of sperm showing some form of abnormal head morphology (Figs. 11 and 12) has been recorded in the different treatment series (Tables 2 and 3). Ch-200 reduced considerably the percentages of sperm with abnormal head morphology and the differences were statistically significant at 7,15, 60, 90 and 120 days, while Ch-30 reduced the occurrence at 60 and 120 days. The feeding of p-DAB alone also produced abnormal sperm in greater number than in the normal control and the differences were statistically significant at all fixation intervals (Tables 2 and 3) while the PB alone group also showed marginally higher incidence of abnormal sperm head as compared to normal controls.

Lipid peroxidation activity—The lipid peroxidation activity (LPO) was increased in the p-DAB + PB fed mice as compared to the normal control at all fixation intervals. However, the activity in the only p-DAB series was also considerably higher than in controls at all fixation intervals while that in the only PB fed mice was not as striking when compared with the normal controls (Tables 4a and 4b). The LPO activity was generally reduced in p-DAB+ PB + Ch-200 treated series though not uniformly in all the tissues as compared to p-DAB + PB treated series. However, interestingly enough, while the lipid peroxidation activity was generally much reduced in the Ch-200 fed mice as compared to Ch-30 fed mice in spleen and kidney, the lower microdose i.e. Ch-30 appeared to reduce the activity in liver more than that of Ch-200 at all fixation intervals (Tables 4a and 4b).The feeding of p-DAB alone also produced marginally increased activity in spleen, kidney and liver at all fixation intervals as compared to normal controls and only PB fed mice.

Alkaline phosphatase activity—The alkaline phosphatase activity (AlAA) in the p-DAB + PB fed mice was highest in spleen and liver at all fixation intervals as compared to normal controls, while the activity considerably declined in both Ch-30 and Ch-200 fed mice at different fixation intervals (Tables 4a and 4b). Ch-200 reduced quite substantially AlAA activity at the early fixation intervals as compared to p-DAB + PB treated mice. However, the protective effect of Ch-30 was not as striking though considerable amount of protection was rendered at 60, 90 and 120 days in some tissues. In the only p-DAB treated mice, the AlAA activity was less as compared to p-DAB + PB, but was considerably higher as compared to normal controls and only PB fed mice.

Table 1—Number of animals showing tumors at different fixation intervals after chronic feeding of PB and/or p-DAB for 7, 15, 30, 60, 90 and 120 days.

Series	No. of specimens used	Tumour incidence 7 days	Tumour incidence 15 days	Tumour incidence 30 days	Tumour incidence 60 days	Tumour incidence 90 days	Tumour incidence 120 days
Normal	45	0/5	0/5	0/5	0/10	0/10	0/10
PB	45	0/5	0/5	0/5	0/10	0/10	0/10
DAB	45	0/5	0/5	0/5	4/10	7/10	9/10
DAB+PB	45	0/5	0/5	0/5	10/10	10/10	10/10
DAB+PB+AL	45	0/5	0/5	1/5	10/10	10/10	10/10
DAB+PB+Ch-30	30	n.a	n.a	n.a	6/10	8/10	9/10
DAB+PB+Ch-200	45	0/5	0/5	0/5	5/10	4/10	4/10
TOTAL	300	0/30	0/30	1/30	35/70	39/70	42/70

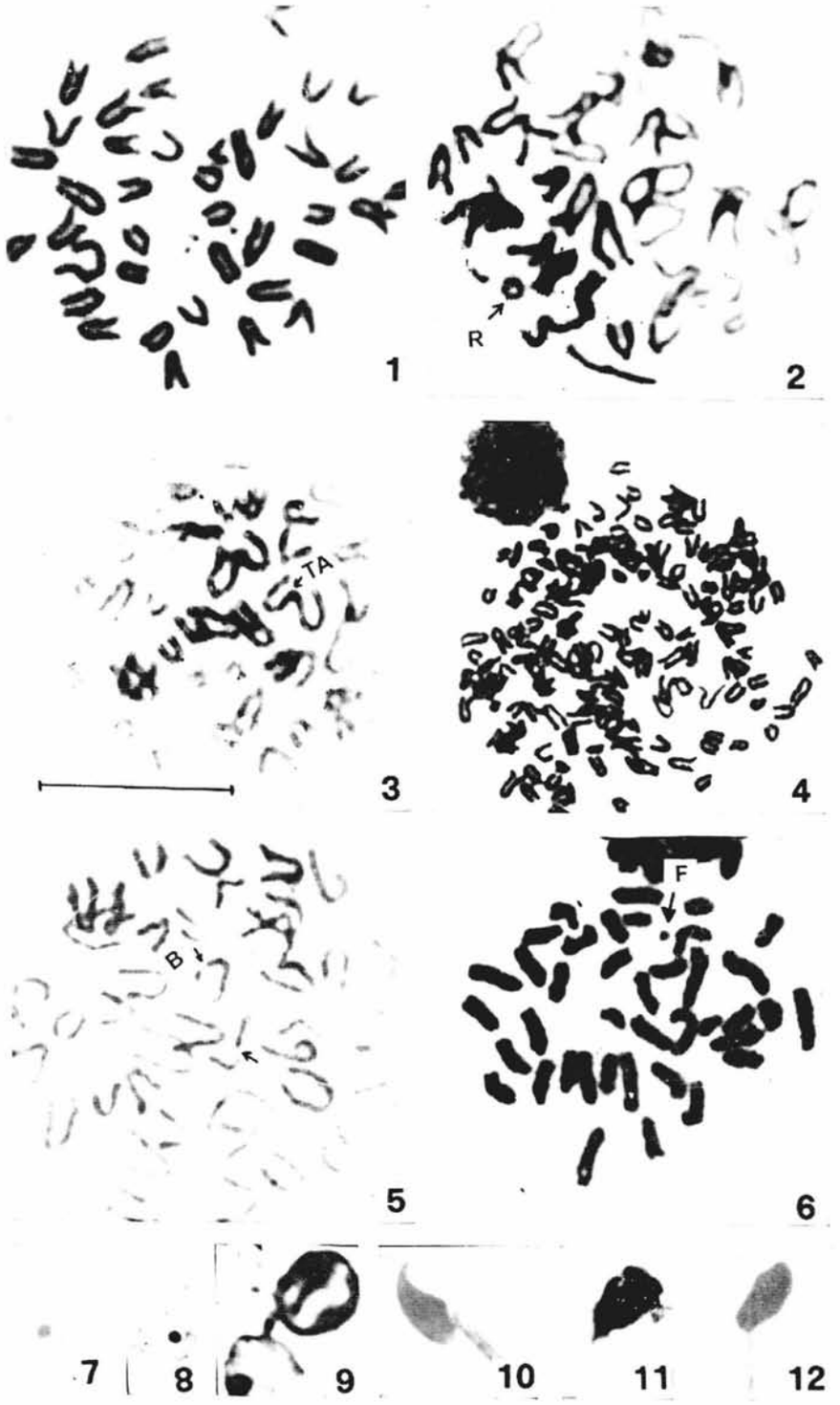
[For longer fixation intervals of 60, 90 and 120 days 10 mice each were used per set; for fixation intervals of 7, 15 and 30 days, 5 mice each were used per set; n.a. = not applicable as experiments not done]

Table 2—Frequency distribution of MI, CA, MN, and SHA in mice of different experimental and control series.

		Mitotic index (MI)		Chromosome aberration (CA)				Micronucleated erythrocytes (MN)					Sperm head anomaly (SHA)	
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.
7	Normal	1.4±0.20	1.0[c]	0.4	1.8	2.2±0.55	3.2[c]	0.13	0.14	0.81	0.14±0.05	0.3[c]	0.94±0.16	0.3[b]
	PB	1.6±0.28	1.1[c]	0.8	2.4	3.2±0.37	6.4[c]	0.28	0.21	0.73	0.24±0.06	0.5[c]	1.08±0.25	0.5[c]
	DAB	2.4±0.36		1.2	4.2	5.4±1.21		0.32	0.47	.0.94	0.40±0.03		1.24±0.18	
	DAB+PB	2.5±0.45	-1.26[a]	1.8	6.8	8.6±0.98	-1.8[n]	0.73	0.63	0.97	0.68±0.06	-0.1[n]	1.46±0.16	-0.46[a]
	DAB+PB +AL	3.76±0.08	0.4n	2.4	8.0	10.4±0.51	2.0[n]	0.77	0.79	0.96	0.78±0.10	0.24[n]	1.92±0.08	0.24[n]
	DAB+PB+Ch-200	2.1±0.68	1.6[a]	1.4	5.2	6.6±1.07	3.8[a]	0.45	0.42	1.09	0.44±0.09	0.34[a]	1.22±0.05	0.70[c]
15	Normal	1.4±0.20	0.9[b]	0.4	1.8	2.20±0.55	3.2[c]	0.13	0.14	0.81	0.14±0.05	0.5[c]	0.94±0.16	0.6[c]
	PB	1.56±0.27	1.26[c]	0.8	3.4	4.20±0.97	7.6[b]	0.49	0.31	0.93	0.40±0.05	0.7[c]	1.2±0.25	1.0[c]
	DAB	2.3±0.35		1.4	4.8	5.4±1.21		0.63	0.61	0.79	0.62±0.15		1.52±0.09	
	DAB+PB	2.66±0.27	-0.4[n]	2.0	7.8	9.80±2.08	-1.0[n]	0.98	0.69	0.75	0.82±0.13	-0.14[n]	1.96±0.07	-0.18[n]
	DAB+PB+AL	2.7±0.27	0.16[n]	2.4	8.4	10.80±0.86	1.6[n]	1.02	0.91	0.76	0.96±0.74	0.14[n]	2.14±0.15	0.38[a]
	DAB+PB+Ch-200	2.5±0.13	0.2[n]	1.4	6.8	8.20±1.66	2.6[n]	0.67	0.68	0.81	0.68±0.04	0.28[n]	1.58±0.13	0.56[a]
30	Normal	1.4±0.2	1.5[c]	0.4	1.8	2.20±0.55	5.4[c]	0.13	0.14	0.81	0.14±0.05	0.4[c]	0.94±0.16	0.9[c]
	PB	1.9±0.27	2.2[c]	1.6	4.4	5.80±1.16	8.6[c]	0.61	0.37	0.86	0.48±0.09	0.9[c]	1.26±0.16	1.2[c]
	DAB	2.94±0.26		2.0	5.8	7.60±0.98		0.64	0.48	1.01	0.56±0.09		1.88±0.11	
	DAB+PB	3.56±0.21	-0.08[n]	2.4	8.4	10.80±1.46	-1.6[n]	0.99	1.12	0.93	1.06±0.07	-0.14[n]	2.14±0.15	-0.32[n]
	DAB+PB+AL	3.64±0.11	0.54[n]	2.6	9.8	12.4±1.63	2.2[a]	1.07	1.34	1.09	1.20±0.2	0.18[n]	2.46±0.15	0.26[n]
	DAB+PB+Ch-200	3.02±0.29	0.62[n]	1.8	6.8	8.60±1.28	3.8[n]	0.85	0.90	0.96	0.88±0.19	0.32[n]	1.88±0.24	0.58[n]

Prot= Protection given by the drug; *P* values: [a]<0.05, [b]<0.01, [c] <0.001, n-non-significant

% protection: row downward: p-DAB vs normal; p-DAB+PB vs normal; p-DAB+PB vs p-DAB +PB + alcohol; p-DAB + PB vs p-DAB + PB + Ch-200. p-DAB + PB + alcohol vs p_DAB + PB + Ch-200.



Figs 1-6—Representative photomicrographs of metaphase complements showing normal set of chromosomes (Fig. 1), and chromosome aberrations like ring (R, Fig. 2), terminal association (TA, Fig. 3), polyploidy (Fig. 4), break (B, Fig. 5), and acentric fragment (F, Fig. 6) in the p-DAB treated series;

Figs 7-9—Showing normo (Figs-7 and 8) and polychromatic erythrocytes (Fig. 9) in the p-DAB treated series;

Figs 10-12—Photomicrographs showing sperm with normal head (Fig-10) in the control and abnormal head morphology (Figs 11 and 12) in the p-DAB treated series. Bar represents 10μm.

Table 3—Frequency distribution of MI, CA, MN, and SHA in mice of different experimental and control series

Fixation intervals (days)	Series	Mitotic index(MI)		Chromosome aberrations(CA)				Micronucleated erythrocytes(MN)					Sperm head anomaly(SHA)	
		% 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.
60	Normal	1.32±0.08	3.9^{c}	1.4	1.4	2.80±0.58	11.6^{c}	0.04	0.26	0.89	0.16±0.05	0.5^{c}	0.44±0.12	2.7^{c}
	PB	2.10±0.48	7.4^{c}	1.6	1.8	3.40±0.98	13.0^{c}	0.14	0.28	0.74	0.16±0.05	0.6^{c}	1.08±0.24	2.2^{c}
	DAB	5.3±0.50		4.8	9.6	14.4±2.87		1.01	0.39	0.97	0.70±0.16		3.18±0.49	
	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+AL	9.1±0.63	-0.4^{n}	5.6	5.8	11.4±2.01	4.4^{b}	0.72	0.94	0.97	0.84±0.129	-0.12^{n}	2.72±0.28	-0.1
	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}
90	Normal	1.32±0.08	4.1^{c}	1.4	1.4	2.80±0.58	8.8^{c}	0.04	0.26	0.89	0.16±0.05	0.9^{c}	0.44±0.12	2.5^{c}
	PB	1.58±0.14	5.1^{c}	2.2	3.0	5.20±0.86	8.0^{c}	0.13	0.22	0.83	0.18 0.08	0.5^{c}	0.52±0.09	2.5^{c}
	DAB	5.42±0.25		5.6	6.0	11.6±0.74		1.16	0.82	0.86	1.03±.018		2.98±0.09	
	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+AL	8.12±0.76	-1.72^{a}	7.0	5.0	12.0±0.84	-1.2^{a}	1.73	1.04	0.86	1.36±0.16	-0.69	3.1±0.11	-0.2
	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}
120	Normal	1.32±0.08	6.6^{c}	1.4	1.4	2.80±0.58	17.0^{c}	0.04	0.26	0.89	0.16±0.05	0.4^{c}	0.44±0.12	1.6^{c}
	PB	2.12±0.30	6.8^{c}	2.4	4.2	6.60±0.93	19.6^{c}	0.21	0.11	0.91	0.16±0.08	0.4^{c}	1.12±0.12	1.1^{c}
	DAB	7.92±0.32		8.0	11.8	19.8±1.02		0.69	0.38	0.94	0.54±0.07		2.02±0.19	
	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 AL	9.86±0.65	-1.66^{n}	15.0	17.8	32.8±1.35	-10.4	1.64	1.34	0.82	1.48±0.15	0.9	3.7±0.07	-2.12
	DAB+PB+Ch-30	8.72±0.65	-0.52	7.8	7.0	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}

Prot = Protection given by the drug; *P* values : ^{a}<0.05, ^{b}<0.01, ^{c}<0.001, n-non-significant

% protection: row downward: p-DAB vs normal; p-DAB+PB vs normal; p-DAB+PB vs p-DAB +PB +alcohol; p-DAB + PB vs p-DAB + PB + Ch-30; p-DAB + PB vs p-DAB + PB + Ch-200

Table 4a—Mean activities of lipid peroxidase (nMol MDA/g wet tissue), acid and alkaline phosphatases (in terms of m Mole phenol liberated/100 mg protein after 30 min of incubation at 37°C), in different organs of mice in treated and control series at early fixation intervals (7,15 and 30 days)

Days		Lipid peroxidase activity						Alkaline phosphatase activity						Acid phosphatase activity					
Interval	Series	Liver activity ± SE	% Prot.	Kidney activity ± SE	% Prot.	Spleen activity ± SE	% Prot.	Liver activity ± SE	% Prot.	Kidney activity ± SE	% Prot.	Spleen activity ± SE	% Prot.	Liver activity ± SE	% Prot.	Kidney activity ± SE	% Prot.	Spleen activity ± SE	% Prot.
7	Normal	9.07±0.55	26.0^{c}	06.10±0.87	37.1^{c}	10.12±1.03	22.5^{c}	0.66± 0.01	0.5^{c}	0.07± 0.004	0.6^{c}	0.09± 0.01	0.03^{a}	0.04± 0.01	0.4^{c}	0.08± 0.004	0.5^{c}	0.10±0.01	0.6^{c}
	PB	22.31± 6.35	26.2^{c}	07.23±0.13	33.4^{c}	15.07±0.82	26.1^{c}	0.52± 0.004	0.5^{c}	0.61± 0.16	1.7^{c}	0.61± 0.001	0.1^{b}	0.17± 0.05	0.7^{c}	0.12± 0.02	0.7^{c}	0.19±0.07	0.7^{c}
	DAB	35.08± 0.36		43.15±0.92		32.65±0.43		0.53± 0.002		0.69± 0.12		0.12± 0.001		0.42± 0.01		0.57± 0.002		0.65±0.002	
	DAB +PB	35.25± 1.77	-7.8^{b}	39.51±0.88	-7.5^{c}	36.20±1.06	-5.4^{b}	0.59± 0.01	0.0	1.75± 0.02	0.04^{n}	0.14± 0.01	0.0	0.74± 0.02	0.0^{n}	0.76± 0.003	-0.0^{n}	0.86±0.001	-0.0^{n}
	DAB +PB +AL	43.05± 0.93	-18.9^{c}	47.06±0.55	7.7^{a}	41.63±1.02	7.5^{n}	0.59± 0.01	0.6^{c}	1.71± 0.12	1.4^{c}	0.14± 0.004	0.12	0.74± 0.01	0.1^{c}	0.77± 0.01	0.5^{c}	0.85±0.003	0.3^{c}
	DAB+PB +Ch200	54.23± 2.25	-11.2^{c}	31.84± 2.42	15.2^{c}	28.69± 4.44	12.9^{a}	0.02± 0.001	0.6^{c}	0.40± 0.001	1.3^{c}	0.02± 0.002	0.12	0.64± 0.01	0.1^{c}	0.29± 0.01	0.5^{c}	0.54±0.001	0.3^{c}
15	Normal	09.07± 0.55	49.4^{c}	05.38± 0.71	36.2^{c}	9.48± 0.88	33.6^{c}	0.09± 0.005	0.5^{c}	0.08± 0.01	1.1^{c}	0.09± 0.01	0.04^{a}	0.09± 0.003	0.4^{c}	0.09± 0.01	0.5^{c}	0.09± 0.01	0.5^{c}
	PB	35.76± 1.75	48.6^{c}	20.57±1.53	29.1^{c}	19.61±4.69	24.4^{c}	0.67± 0.03	0.5^{c}	0.04± 0.02	1.7^{c}	0.77± 0.03	0.1^{b}	0.29± 0.02	0.6^{c}	0.17± 0.03	0.7^{c}	0.35± 0.02	0.7^{c}
	DAB	58.44± 2.23		41.57±0.36		43.05±1.64		0.54± 0.01		1.18± 0.01		0.13± 0.003		0.47± 0.03		0.63± 0.01		0.61± 0.004	
	DAB +PB	57.66±. 0.56	12.01^{b}	34.51±4.17	-2.9^{n}	33.84±1.80	-9.1^{n}	0.57± 0.005	-0.04^{n}	1.73± 0.04	-0.1^{n}	0.15± 0.004	-0.01^{n}	0.73± 0.02	-0.1^{b}	0.78± 0.01	-0.0^{c}	0.78± 0.01	-0.0^{c}
	DAB +PB +AL	69.67± 0.18	36.2^{b}	31.53±0.66	24.4^{c}	42.92±6.41	-5.3^{n}	0.61± 0.01	0.1^{c}	1.86± 0.01	0.2^{c}	0.16± 0.01	0.02^{n}	0.79± 0.002	0.3^{c}	0.79±0.001	0.2^{c}	0.82± 0.002	0.1^{c}
	DAB+PB +Ch200	21.42± 8.05	48.3^{c}	10.15±1.11	21.4^{c}	39.17±7.49	3.7^{n}	0.51± 0.01	0.1^{c}	1.49± 0.02	0.4^{c}	0.13± 0.01	0.03^{a}	0.47± 0.004	0.3^{c}	0.56± 0.002	0.2^{c}	0.67± 0.01	0.2^{c}

Contd.

Table 4a—(Contd.)

Days Interval	Series	Lipid peroxidase activity						Alkaline phosphatase activity						Acid phosphatase activity					
		Liver activity ± SE	% Prot.	Kidney activity ± SE	% Prot.	Spleen activity ± SE	% Prot.	Liver activity ± SE	% Prot.	Kidney Activity ± SE	% Prot.	Spleen activity ± SE	% Prot.	Liver activity ± SE	% Prot.	Kidney activity ± SE	% Prot.	Spleen activity ± SE	% Prot.
30	Normal	06.92± 1.03	80.8^{c}	07.38±0. 92	80.4^{c}	9.38±0.66	59.6^{c}	0.05± 0.01		0.06± 0.01	0.8^{c}	0.08± 0.01	1.2^{c}	0.08± 0.01	0.6^{c}	0.09± 0.01	0.6^{c}	0.09± 0.01	0.9^{c}
	PB	45.69± 1.47	80.9^{c}	45.69±1. 47	80.5^{c}	59.67±1.29	59.4^{c}	0.43± 0.07		0.43± 0.05	0.7^{c}	0.77± 0.06	0.4^{c}	0.19± 0.02	0.7^{c}	0.43± 0.06	0.6^{c}	0.56± 0.06	0.7^{c}
	DAB	87.74± 1.51		87.74±1. 52		68.97±0.55		1.03± 0.01		0.86± 0.03		1.28± 0.02		0.66± 0.001		0.71± 0.001		0.99± 0.01	
	DAB +PB	87.89± 2.98	18.9^{c}	87.89±2. 98	-27.3^{c}	68.75±0.89	-21.3^{c}	0.94± 0.07	-0.1^{n}	1.12± 0.03	-0.2^{b}	1.13± 0.01	-0.4^{c}	0.85± 0.001	0.1^{c}	0.72± 0.002	-0.3^{c}	0.78± 0.004	-0.2^{c}
	DAB +PB +AL	69.02± 1.64	47.9^{c}	60.61±1. 38	41.9^{c}	90.01±2.94	-23.0^{c}	0.99± 0.22	0.7^{c}	1.28± 0.01	0.7^{c}	1.57± 0.01	-0.2^{n}	0.74± 0.013	0.2^{c}	0.98± 0.01	0.3^{c}	0.93± 0.01	0.3^{C}
	DAB+PB +Ch200	39.99± 0.54	29.03^{n}	45.99±1. 17	14.6^{n}	91.79±0.76	1.7^{n}	0.23± 0.04	0.8^{c}	0.38± 0.02	0.9^{c}	1.3± 0.01	-0.3^{c}	0.67± 0.03	0.1^{n}	0.39± 0.002	0.6^{c}	0.50± 0.004	0.4^{c}

Prot. = Protection given by the drug; % protection: row downward: p-DAB vs normal; p-DAB+PB vs normal; p-DAB+PB vs p-DAB +PB +alcohol; p-DAB + PB vs p-DAB + PB + Ch-30; p-DAB + PB vs p-DAB + PB + Ch-200; *P* values: ^{a}<0.05; ^{b}<0.01; ^{c}<0.001, n-non-significant

Table 4b—Mean activities of lipid peroxidase (nmole MDA/g wet tissue), acid and alkaline phosphatase activities (in terms of mmole phenol liberated/100 mg protein after 30 min of incubation at 37°C), in different organs of treated and control series at longer fixation intervals (60, 90 and 120 days)

Days interval	Series	Lipid peroxidase activity						Alkaline phosphatase activity						Acid phosphatase activity					
		Liver activity ± SE	% Prot.	Kidney activity ± SE	% Prot.	Spleen activity ± SE	% Prot.	Liver activity ± SE	% Prot.	Kidney activity ± SE	% Prot.	Spleen activity ± SE	% Prot.	Liver activity ± SE	% Prot.	Kidney activity ± SE	% Prot	Spleen activity ± SE	% Prot
60	Normal	18.72 ±1.27	61.5[c]	16.67 ±0.26	41.9[c]	25.44 ±0.68	46.0[c]	0.09± 0.01	0.7[c]	0.12 ±0.03	0.6[c]	0.09 ±0.01	1.02[c]	0.10 ±0.002	0.8[c]	0.09 ±0.004	0.9[c]	0.10 ±0.003	1.05[c]
	PB	16.58 ±0.43	98.3[c]	15.85 ±1.39	117.7[c]	17.35 ±0.45	182.5[c]	0.11± 0.001	0.2[c]	0.11 ±0.004	0.1[b]	0.11 ±0.002	0.1[a]	0.18 ±0.02	0.2[c]	0.15 ±0.02	0.2[c]	0.15 ±0.02	0.2[c]
	DAB	80.25± 12.21		58.61± 0.97		71.47± 1.66		0.77± 0.00		0.76±0.0 04		1.11±0. 05		0.89± 0.01		1.05± 0.01		1.15± 0.01	
	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+AL	71.44± 2.28	46.04	69.49± 2.26	64.9[c]	91.33± 1.37	115.6[c]	0.71± 0.03	-0.43	0.47± 0.01	-0.22	1.29±0. 07	-1.14	1.03± 0.01	-0.73	1.09± 0.04	-0.8	1.44± 0.015	-1.17
	DAB+PB+ Ch30	24.26 ±0.78	93.2[a]	29.04 ±1.05	105.4[c]	44.32 ±1.14	163.6[b]	0.11 ±0.03	0.17[b]	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	32.8	19.737± 0.45	114.7[c]	48.71 ±3.28	159.2[b]	0.05 ±0.03	0.22[b]	0.28 ±0.04	-0.03	0.04 ±0.01	0.11[c]	0.24 ±0.03	0.06	0.22 ±0.04	0.07	0.30 ±0.03	0.03
90	Normal	18.72 ±1.27	46.8[c]	15.85 ±1.39	23.02[c]	25.44 ±0.68	51.2[c]	0.09 ±0.01	0.7[c]	0.12 ±0.03	0.7[c]	0.09± 0.01	0.3[c]	0.10 ±0.002	0.6[c]	0.09 ±0.004	0.7[c]	0.10 ±0.003	1.04[c]
	PB	25.21 ±0.31	50.5[c]	16.58 ±0.62	45.6[c]	21.88 ±0.98	66.5[c]	0.11 ±0.002	0.2[c]	0.12 ±0.003	0.2[b]	0.11± 0.003	0.1[b]	0.19 ±0.003	0.3[c]	0.18 ±0.01	0.2[c]	0.18 ±0.003	0.1[c]
	DAB	65.48± 2.32		38.87± 0.65		76.66± 1.29		0.77± 0.002		0.79± 0.02		0.38± 0.001		0.69± 0.02		0.82± 0.03		1.14± 0.02	
	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 +AL	94.66± 5.68	-25.2	51.89± 3.25	9.53	78.20± 0.429	13.8[c]	0.74± 0.02	-0.5	0.92± 0.001	-0.65	0.41± 0.01	-0.24	1.45± 0.13	-1.09	0.79± 0.04	0.49	1.58± 0.02	-1.34
	DAB+PB+ Ch30	48.77 ±3.07	20.5[b]	22.12 ±0.76	39.3[c]	30.95 ±3.36	61.04[c]	0.04 ±0.01	0.19[b]	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	-66.5	22.18 ±0.64	39.24[b]	30.49 ±0.75	61.51[c]	0.14 ±0.06	0.09	0.34 ±0.02	-0.07	0.09± 0.04	0.08	0.30 ±0.04	0.06	0.29 ±0.02	0.01	0.30 ±0.04	0.06
120	Normal	18.72 ±1.27	60.2[c]	15.85 ±1.39	77.4[c]	25.435± 0.68	37.8[c]	0.09 ±0.01	0.7[c]	0.12 ±0.03	0.5[c]	0.09± 0.01	0.11[c]	0.10 ±0.002	0.9[c]	0.09 ±0.004	0.6[c]	0.10 ±0.003	0.9[c]

Contd.

Table 4b—(Contd.)

Days interval	Series	Lipid peroxidase activity						Alkaline phosphatase activity						Acid phosphatase activity					
		Liver activity ± SE	% Prot.	Kidney activity ± SE	% Prot.	Spleen activity ± SE	% Prot.	Liver activity ± SE	% Prot.	Kidney activity ± SE	% Prot.	Spleen activity y ± SE	% Prot.	Liver activity ± SE	% Prot.	Kidney activity ± SE	% Prot	Spleen activity ± SE	% Prot
	PB	38.63 ±2.79	146.7[c]	44.96 ±1.49	122.8[c]	28.72 ±0.65	249.1[c]	0.14 ±0.003	0.1[b]	0.12 ±0.01	0.2[b]	0.17± 0.002	0.1[a]	0.20 ±0.001	0.3[c]	0.15 ±0.01	0.3[c]	0.18 ±0.001	0.2[c]
	DAB	78.87± 0.39		93.33± 1.24		63.28± 0.25		0.79± 0.001		±0.660.0 01		0.203±0. 001		1.05± 0.02		0.71± 0.00		1.01± 0.001	
	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 +AL	69.28± 0.75	-86.5	68.92± 1.43	69.08[c]	43.58± 0.79	230.9[c]	0.902±0. 002	-0.71	0.73± 0.002	-0.42	0.26± 0.001	0.11	0.69± 0.02	-0.29	0.64±0.005	-0.3	0.75± 0.01	-0.46
	DAB+PB+ Ch30	113.04± 1.14	52.4[a]	89.79 ±0.5	48.9	92.56 ±0.18	181.9[c]	0.19 ±0.003	0.001	0.27 ±0.05	0.04	0.15± 0.003	0.01	0.50 ±0.05	0.11	0.67 ±0.09	0.33	0.22 ±0.05	0.06
	DAB+PB+ Ch200	190.25± 0.48	-25.8	18.21 ±0.5	120.5[b]	110.51± 0.36	160.1[c]	0.08 ±0.01	0.102[c]	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[b]	0.24 ±0.01	0.05

Prot. = Protection given by the drug; % protection: row downward: p-DAB vs normal; p-DAB+PB vs normal; p-DAB+PB vs p-DAB +PB +Alcohol; p-DAB + PB vs p-DAB + PB + Ch-30; p-DAB + PB vs p-DAB + PB + Ch-200; *P* values [a]<0.05; [b]<0.01; [c]<0.001

Acid phosphatase activity—The acid phosphatase activity (AcAA) in spleen, liver and kidney was very high in p-DAB + PB fed mice as compared to normal controls. The activity was only marginally reduced in the Ch-30 fed mice at some fixation intervals (Tables 4a and 4b), but the differences were not statistically significant. However, in the Ch-200 fed series, there was significant decrease in the AcAA activity at all fixation intervals, showing the greater protective efficacy of Ch-200. In the p-DAB alone treated mice, the activity was considerably higher than that of normal control and PB treated series.

Glutamate oxaloacetate and pyruvate transaminase activities—The GOT activities in the p-DAB + PB fed series were appreciably enhanced in all tissues at all fixation intervals as compared to normal controls. The activity was only marginally enhanced in the p-DAB alone and PB alone series as compared to normal controls (Tables 5a and 5b). The GOT activities in liver of p-DAB + PB fed mice also fed either Ch-200 were decreased at 7, 15 and 30 days, but subsequently increased considerably at both 60 and 90 days, but again significantly decreased at 120 days as compared to mice only fed p-DAB + PB. In the kidney tissue, however, the GOT activities were decreased at 7, 30, 60 and 90 days, but increased non-significantly at 120 days. In the spleen, there was an increase at 7, 60 days, but the activity was decreased at 15, 30, 90 and 120 days. In the p-DAB alone treated mice, the activity was generally higher than in the normal controls.

In the Ch-30 fed series, the GOT activities increased in liver at 60 days, but decreased quite appreciably at 90 and 120 days. On the other hand, in kidney the activities were decreased in all the intervals. In spleen, the activity increased to some extent at 60 days, but later on decreased at both 90 and 120 days. In the p-DAB alone treated mice, the GOT activity was generally increased at all fixation intervals as compared to normal controls.

In the p-DAB plus PB plus alcohol fed series, there was an increase of GOT activity at 60 days as compared to only p-DAB + PB fed series. The activity was subsequently decreased at both 90 and 120 days.

The GPT activities in liver of p-DAB + PB fed mice increased appreciably in all tissues at all fixation intervals as compared to normal and only PB fed series (Tables 5a and 5b).

In the mice fed Ch-200 along with p-DAB + PB, the GPT activities were substantially decreased at 7, 15. and 30 days but subsequently increased at 60 days; the activities were again decreased at both 90 and 120 days as compared to mice fed with p-DAB + PB. In kidney, the activity was decreased at all intervals except at 120 days. In spleen, the activity was reduced at 7, 15 and 30 days, did not appreciably change at 60 days, but subsequently the activity was quite appreciably lowered again at 90 and 120 days.

In the p-DAB + PB plus alcohol fed series, the GPT activities in liver were enhanced at 7, 15, 30 and 90 days, but decreased to some extent at 60 and 120 days as compared to p-DAB plus PB fed series. In kidney, the activity was slightly increased at 7, 15, 60, 90 days followed by a slight decrease again at 120 days. In spleen, the activities were decreased at 7, were more or less same at 15, 30 and 60 days, but the activities appreciably increased at both 90 and 120 days.

Discussion

Dietary PB had positive carcinogenic effect when fed with the azo dye 2-methyldiaminoazobenzene, but neither of these two when fed alone showed positive hepatocarcinogenesis in both mice and rat[14-16]. However, in the present study, 20 out of 45 mice fed p-DAB alone yielded livers with signs of tumor nodules at the longer intervals. Further, 7 out of 30 mice in the Ch-30 fed and 17 out of 30 mice in the Ch-200 fed series, that is, 24 out of 75 mice or about 32% of mice that received both p-DAB and PB along with either microdose of Chelidonium did not develop tumors in liver, while all other mice fed p-DAB + PB but no Chelidonium developed tumors. Actually this is a remarkable finding as neither of these microdoses had literally a single molecule of original active ingredient (ethanolic extract of the plant) in their highly diluted forms and were yet capable of reducing/delaying tumor growth in mice. Such agents that can somehow 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. Incidentally, such protection against tumorigenesis/carcinogenesis has been alternatively achieved in some other ways e.g. against oxidative DNA damage[17], by developing genetic resistance[18], by hormonal manipulation and ectomy of endocrine glands[19,20], administration of vitamins[21] etc. Attempts on favorably modulating cytogenetic and biochemical effects in chemically induced liver cancer in mice by any such microdoses do not seem to have been recorded earlier although Roberfroid *et al*[22] reported

Table 5a—Mean activities of GOT and GPT in different organs (expressed as mM/minute/mg protein) of treated and control mice at early fixation intervals (7,15 and 30 days)

Days interval	Series	GOT						GPT					
		Liver		Kidney		Spleen		Liver		Kidney		Spleen	
		Activity ± SE	% Prot.	Activity ± SE	% Prot.	Activity ± SE	% Prot.	Activity ± SE	% Prot	Activity ± SE	% Prot	Activity ± SE	% Prot.
7	Normal	0.09± 0.01	0.6^{c}	0.17± 0.02	0.6^{c}	0.06± 0.01	0.1^{b}	0.03± 0.01	0.59^{c}	0.03± 0.01	0.3^{c}	0.03± 0.003	0.1^{c}
	PB	0.31± 0.01	0.4^{c}	0.56± 0.04	0.2^{c}	0.21± 0.02	0.2^{c}	0.33± 0.01	0.2^{b}	0.03± 0.01	0.1^{b}	0.13± 0.02	0.1^{c}
	DAB	0.64± 0.03		0.72± 0.06		0.16± 0.01		0.62± 0.01		0.29± 0.05		0.08± 0.01	
	DAB +PB	0.53± 0.01	0.0^{n}	0.41±0.06	0.03^{n}	0.44± 0.01	-0.1^{n}	0.50± 0.01	-0.1^{c}	0.12± 0.01	-0.0^{n}	0.16± 0.003	0.0^{n}
	DAB+PB+AL	0.53± 0.01	0.1^{c}	0.38± 0.01	-0.3^{n}	0.30± 0.004	0.2^{c}	0.63± 0.001	0.4^{c}	0.13± 0.002	0.1^{c}	0.15± 0.004	0.1^{c}
	DAB+PB+Ch200	0.45± 0.002	0.1^{c}	0.70± 0.004	-0.3^{n}	0.26± 0.01	0.0^{b}	0.14± 0.001	0.5^{c}	0.05± 0.001	0.1^{c}	0.11± 0.001	0.0^{c}
15	Normal	0.08± 0.01	1.1^{c}	0.11± 0.03	0.7^{c}	0.05± 0.01	0.4^{c}	0.03±0.01		0.03±0.004	0.1^{c}	0.04±0.004	0.1^{c}
	PB	0.51± 0.01	0.2^{c}	0.55± 0.03	0.9^{c}	0.22± 0.02	0.4^{c}	0.27±0.01		0.08±0.02	0.2^{c}	0.09±0.01	0.1^{b}
	DAB	1.18± 0.02		0.76±0.02		0.44± 0.01		0.93±0.01		0.15±0.002		0.11±0.002	
	DAB +PB	0.69± 0.01	-0.1^{c}	0.97± 0.01	-0.3^{c}	0.44± 0.01	0.0^{n}	0.48±0.002	-0.1^{c}	0.19±0.004	-0.1^{c}	0.095±0.004	0.0^{n}
	DAB+PB+AL	0.79± 0.01	0.3^{c}	1.24±0.01	0.1^{c}	0.45± 0.01	0.1^{a}	0.55±0.003	0.1^{c}	0.24±0.003	0.03^{c}	0.09±0.001	0.0^{c}
	DAB+PB+Ch200	0.37± 0.01	0.4^{c}	0.84±0.02	0.4^{c}	0.39± 0.01	0.1^{b}	0.38±0.01	0.2^{c}	0.16±0.01	0.1^{c}	0.08±0.003	0.0^{c}
30	Normal	0.05± 0.01	0.9^{c}	0.04± 0.01	3.2^{c}	0.04± 0.004	0.4^{c}	0.05±0.01		0.04±0.003	0.1^{c}	0.04±0.007	0.2^{c}
	PB	0.72± 0.01	0.5^{c}	0.47±0.01	3.2^{c}	0.26± 0.04	0.4^{c}	0.41±0.03		0.06±0.01	0.4^{c}	0.06±0.01	0.1^{c}
	DAB	1.04± 0.01		3.21± 0.02		0.41± 0.004		0.76±0.01		0.17±0.004		0.25±0.003	
	DAB+PB	1.20± 0.01	-0.1^{c}	3.21± 0.02	2.1^{c}	0.41±0.004	-0.1^{n}	0.81±0.01	-0.0^{n}	0.42±0.01	0.3^{c}	0.15±0.003	0.0^{n}
	DAB+PB+AL	1.28± 0.003	0.5^{c}	1.08± 0.01	1.9^{c}	0.35± 0.003	0.2^{c}	0.84±0.01	0.1^{c}	0.17±0.002	0.3^{c}	0.15±0.14	0.1^{c}
	DAB+PB+Ch200	0.69± 0.013	0.6^{c}	1.26± 0.01	-0.2^{c}	0.17± 0.002	0.2^{c}	0.70±0.01	0.1^{c}	0.12±0.003	0.1^{c}	0.05±0.002	0.1^{c}

Prot. = Protection given by the drug; % protection: row downward: p-DAB vs normal; p-DAB+PB vs normal; p-DAB+PB vs p-DAB +PB +alcohol; p-DAB + PB vs p-DAB + PB + Ch-30; p-DAB + PB vs p-DAB + PB + Ch-200; *P* values ^{a}<0.05; ^{b}<0.01; ^{c}<0.001, n-non-significent

Table 5b—Mean activities of GOT and GPT in different organs (expressed as mM/minute/mg protein) of treated and control mice at longer fixation intervals (60, 90 and 120 days).

Days Interval	Series	GOT						GPT					
		Liver		Kidney		Spleen		Liver		Kidney		Spleen	
		Activity ± SE	% Prot	Activity ± SE	% Prot	Activity ± SE	% Prot.	Activity ± SE	% Prot	Activity ± SE	% Pro.	Activity ± SE	% Prot
60	Normal	0.07±0.02	0.5^{c}	0.06±0.03	0.5^{c}	0.06±0.01	0.5^{c}	0.06±0.01	0.4^{c}	0.04±0.01	0.1^{b}	0.04±0.01	0.1^{c}
	PB	0.29±0.002	0.5^{c}	0.42±0.04	1.8^{c}	0.19±0.04	0.1^{c}	0.47±0.02	0.5^{c}	0.19±0.03	0.2^{c}	0.08±0.01	0.02^{n}
	DAB	0.60±0.01		0.54±0.004		0.55±0.01		0.45±0.01		0.09±0.01		0.16±0.01	
	DAB+PB	0.54±0.01	-0.3^{a}	1.88±0.01	0.8^{a}	0.19±0.004	-0.0^{n}	0.55±0.04	0.0^{n}	0.19±0.01	0.0^{n}	0.06±0.002	0.0^{n}
	DAB+PB+AL	0.79±0.03	-0.1^{n}	1.07±0.12	1.2^{c}	0.22±0.003	-0.0^{n}	0.55±.001	-0.0^{n}	0.19±0.01	0.1^{b}	0.06±0.003	0.0^{n}
	DAB+PB+Ch30	0.66±0.006	-0.6^{b}	0.64±0.02	1.4^{c}	0.22±0.04	-0.0^{n}	0.58±0.02	-0.1^{a}	0.12±0.001	0.1^{c}	0.06±0.025	0.0^{n}
	DAB+PB+Ch200	1.09±0.02		0.51±0.01		0.21±0.004		0.69±0.01		0.08±0.01		0.06±0.002	
90	Normal	0.07±0.01	1.7^{c}	0.06±0.01	0.6^{c}	0.01±0.003	0.4^{c}	0.04±0.004	1.3^{c}	0.03±0.002	1.3^{c}	0.05±0.01	0.1^{b}
	PB	0.34±.001	0.7^{c}	0.33±0.02	0.8^{c}	0.32±0.004	0.4^{c}	0.35±0.001	0.6^{c}	0.13±0.003	0.2^{c}	0.13±0.00	0.1^{b}
	DAB	1.79±0.02		0.66±0.01		0.42±0.01		1.33±0.01		1.36±0.01		0.12±0.004	
	DAB+PB	0.77±0.01	0.1^{a}	0.88±0.02	-0.1^{n}	0.41±0.03	-0.1^{n}	0.61±0.01	-0.4^{b}	0.19±0.01	-0.0^{n}	0.12±0.003	-0.1^{n}
	DAB+PB+AL	0.67±0.02	0.2^{a}	0.99±0.02	0.8^{c}	0.55±0.07	0.1^{a}	0.99±0.003	0.0^{n}	0.22±0.02	0.0^{n}	0.17±0.01	0.0^{b}
	DAB+PB+Ch30	0.62±0.002	-0.2^{a}	0.06±0.003	0.2^{b}	0.36±0.004	0.1^{a}	0.62±0.003	-0.2^{a}	0.18±0.003	0.1^{b}	0.08±0.004	0.0^{n}
	DAB+PB+Ch200	0.92±0.01		0.64±0.10		0.34±0.02		.079±0.003		0.13±0.01		0.10±0.003	
120	Normal	0.05±0.004	0.6^{c}	0.06±0.01	0.7^{c}	0.03±0.003	0.4^{c}	0.33±0.01	0.2^{c}	0.04±0.01	0.1^{c}	0.03±0.004	0.1^{c}
	PB	0.38±0.01	0.8^{c}	0.36±0.02	0.7^{c}	0.26±0.03	0.3^{c}	0.29±0.004	04^{c}	0.07±0.003	0.1^{c}	0.07±0.01	0.1^{c}
	DAB	0.68±0.01		0.80±0.003		0.47±0.003		0.55±0.002		0.17±0.01		0.14±0.003	
	DAB +PB	0.87±0.001	0.1^{a}	0.79±0.001	0.1^{n}	0.33±0.002	-0.1^{n}	0.69±0.01	0.1^{a}	0.18±0.004	0.0^{n}	0.11±0.003	0.1^{a}
	DAB +PB+AL	0.76±0.003	0.8^{c}	0.68±0.003	0.01^{n}	0.43±0.004	0.01^{a}	0.61±0.002	0.1^{a}	0.16±0.002	-0.0^{n}	0.16±0.004	0.0^{a}
	DAB+PB+Ch30	0.06±0.300	0.1^{c}	0.78±0.021	-0.5	0.32±0.010	0.1^{a}	0.58±0.002	0.1^{b}	0.21±0.003	-0.1^{c}	0.07±0.03	0.0^{b}
	DAB+PB+Ch200	0.79±0.002		1.26±0.004		0.23±0.001		0.63±0.01		0.27±0.01		0.08±0.001	

Prot. = Protection given by the drug; % protection: row downward: p-DAB vs normal; p-DAB+PB vs normal; p-DAB+PB vs p-DAB +PB +Alcohol; p-DAB + PB vs p-DAB + PB + Ch-30; p-DAB + PB vs p-DAB + PB + Ch-200; *P* values: $^{a}<0.05$; $^{b}<0.01$; $^{c}<0.001$, n-non-significant

that micro doses of PB 9C positively reduced the incidence of tumors and mortality in rats chronically fed with another carcinogen acetylaminofluorene along with PB. These authors, however, only restricted their study to recording the incidence of tumor formation and mortality and did not consider any of the protocols used in the present study.

The observation of reduced frequencies of chromosome aberrations, MN and abnormal sperm head at different fixation intervals in the drug fed group of mice was also significant from the point of view of concomitant reduced number of tumor in this group. Increase in number of chromosomal and nuclear damages is generally associated with and implicated to progression of liver toxicity and tissue necrosis[23]. There were supporting evidences of detoxification in terms of activities of several marker enzymes in various organs like liver, spleen and kidney. 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[24-27]. Although glutathione activity has not been studied, there were definite favorable modulations observed in the activities of lipid peroxidase, acid and alkaline phosphatases in both p-DAB + PB + Ch-30 or Ch-200 fed series as compared to p-DAB + PB fed mice series. Further, the positive changes in activities of the other toxicity marker enzymes like GOT and GPT in liver, spleen and kidney of the mice fed p-DAB + PB + Ch-30 or Ch-200 would also speak for hepatoprotective effect rendered by the microdoses of Chelidonium against p-DAB + PB induced hepatocarcinogenesis. In fact, the serum levels of GOT and GPT have also been found to become elevated whenever disease process affects liver cell integrity[28]. Increases of both the transaminases are a common finding in liver diseases with GPT often being higher than GOT[29]. Thus, the favorable modulations of some of these enzymes in the Chelidonium fed mice as compared to drug unfed p-DAB + PB fed mice along with less amount of other genotoxic damages were strongly suggestive that the microdoses of Chelidonium i.e. Ch-30 and Ch-200 had positive protective effects against the hepatocarcinoma induced by p-DAB along with PB, and that Ch-200 appeared to have marginally better effects at the longer intervals.

Incidentally, DAB and its metabolites have been reported to cause oxidative DNA damage[30], which could also be attributable to the various types of chromosome aberrations encountered in the bone marrow cells of mice treated with p-DAB individually and in combination with PB. The formation of adducts, DNA-copper-hydroperoxo complexes, etc as suggested by Ohnishi *et al*[30] could also play an important role in the carcinogenic processes of DAB.

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 demonstrated in the present study. Since the microdoses used were diluted much beyond Avagadro's limit, there could hardly be any original molecule of the drug substance left in them. So in the absence of any original drug molecule (and yet showing positive modulations), the pathway or molecular mechanism of action of the drugs can not possibly be traced in the same way as those of other drugs with macromolecular constituents. Therefore, any attempt to explain the mechanism of action of these highly diluted drugs will have to be rather speculative at the moment. However, since there was unmistakable cause-effect manifestation, there also must be a comprehensive mechanism presently unknown or which at best can be hypothesized. These drugs possibly send specific signals in the receptor cells that could activate specific hypothalamic region of brain in a manner that could possibly help elicit further signals to activate or repress certain transcriptional activities to restore the damages caused due to the carcinogen. One way to test this hypothesis can be either to explore signal transduction pathways or else to measure specific transcription factors with suitable experimental design. Incidentally, Khuda-Bukhsh[31] proposed a hypothesis to explain the possible mechanism of action of such micro doses based on many circumstantial evidences [32-37] that the micro doses act through the regulation of relevant gene expression by eliciting impulses similar to that of some hormones and enzymes. The failure to show antimutagenic efficacy of some homeopathic drugs after simultaneous administration of Actinomycin D, a transcription blocker, also lends support to this contention[34,36]. This hypothesis can suitably explain the mechanisms involved in the repair of damaged chromosomes or sperm head by the application of the microdoses. This can also explain the antagonizing action against tumorigenesis/carcinogenesis, as observed in the present study. Since the transformation and 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 suppressor genes, it may be speculated that the microdoses of the drug may have interfered with the process of carcinogenesis by modifying action of certain of these genes responsible for the transformation of cells to cancerous ones.

In fact the modulating effects of the drug on apparent recovery of altered genomic changes would further lead one to believe 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. However, a more thorough and in-depth study with electron microscopy to ascertain other structural alterations, if any, at the membrane and sub-cellular levels would be warranted to understand more precisely the exact molecular mechanisms of action of the microdoses of *Chelidonium majus* in mammalian system *in vivo*.

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