THE JOURNAL OF ALTERNATIVE AND COMPLEMENTARY MEDICINE
Volume 11, Number 5, 2005, pp. 839–854
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Efficacy of the Potentized Homeopathic Drug, Carcinosin 200, Fed Alone and in Combination with Another Drug, Chelidonium 200, in Amelioration of p-Dimethylaminoazobenzene–Induced Hepatocarcinogenesis in Mice

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ABSTRACT

Objectives: This study was conducted to examine whether the potentized homeopathic remedy Carcinosin 200, fed alone and in combination with Chelidonium 200, has differential protective effects against p-dimethylaminoazobenzene (p-DAB)–induced hepatocarcinogenesis in mice.

Design: Liver tumors were induced in mice through chronic feeding of p-DAB (initiator) and phenobarbital (PB, promoter). The mice were divided into two subgroups: (1) one was fed potentized Alcohol 200 and served as controls; and (2) the other was fed Carcinosin 200 alone or in combination with Chelidonium 200 and divided into several sets. The relative efficacy of the two potentized remedies, alone or in combination, in combating hepatocarcinogenesis was assessed through several cytogenetical endpoints such as chromosome aberrations, induction of micronuclei, sperm head anomaly, and mitotic index at several intervals of fixation (days 7, 15, 30, 60, 90, and 120). Several toxicity biomarkers such as acid and alkaline phosphatases, glutamate oxaloacetate transaminase, glutamate pyruvate transaminase, and lipid peroxidation activity were also assayed in three organs of treated and control mice. In addition, recovery by the homeopathic drugs, if any, of tissue damage inflicted because of chronic feeding of p-DAB and PB was also assessed by optical, scanning, and transmission electron microscopies of liver done at days 60 and 120.

Results: Both Carcinosin 200 and Chelidonium 200 when administered alone show considerable ameliorative effect against p-DAB–induced hepatocarcinogenesis in mice; but the conjoint feeding of these two drugs appears to have had a slightly greater protective effect.

Conclusions: These homeopathic remedies have the potential to be used as complementary and alternative medicine in liver cancer therapy, particularly as supporting palliative measures.

INTRODUCTION

Feeding of carcinogenic azo dyes such as p-dimethylaminoazobenzene (p-DAB) produces liver damage with neoplastic characteristics.[1] Dietary phenobarbital (PB) also has a positive carcinogenic effect only when fed conjointly with p-DAB in both mice and rats.[2,3]

In continuation of earlier works[4,5] the present paper reports the relative efficacy of a potentized homeopathic drug, Carcinosin 200 (Car 200), administered alone and in combination with Chelidonium 200 (Ch 200), in amelioration of p-DAB–induced hepatocarcinogenesis in mice. In homeopathy, various potencies of Chelidonium are routinely used against several forms of liver ailments, including liver cancer. Car 200 is also used as an intermittent medicine, particularly when malignancy is suspected. Carcinosin is believed to have the ability to modify favorably "symptoms in all cases in which a history of carcinoma can be elicited or

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symptoms of the disease itself exist."[6] However no systematic study seems to have been carried out in any mammalian model to examine whether Car 200 can really act favorably as does Ch 200,[4,5] or can enhance or inhibit ameliorating effects of Ch 200, when administered conjointly, against p-DAB–induced hepatocarcinogenesis in mice. The present study was undertaken to address these questions.

METHODS

A group of 45 healthy mice (Swiss albino *Mus musculus*, ethically maintained) weighing between 25 and 30 g were used for each of the treated and control series for six fixation intervals for 7, 15, 30, 60, 90, and 120 days (Table 1). The group was divided into six sets, as follows. (1) The first set of mice were allowed normal diet and water *ad libitum* and served as normal controls (C). (2) The second set were fed the same diet as in first set plus 0.06% p-DAB (D-6760, Sigma Chemical, St. Louis, MO), a known "initiator" of hepatocarcinoma, was added to it and the water was replaced with 0.05% aqueous solution of PB, a known "promoter," until sacrificed. (3) The third set were given p-DAB and PB in the same way as in the previous group but were also fed 0.06 mL of stock solution of the drug Ch 200 twice a day (6 AM and 6 PM) from day 1 onward of p-DAB feeding. (4) In the fourth set, the feeding of Car 200 (0.06 mL) was made along with p-DAB and PB once a day (12 noon) all along until sacrificed. (5) The fifth set were fed as in group 2 along with Ch 200 (at 6 AM and 6 PM) and Car 200 (once at 12 noon). (6) The sixth set were fed as in group 2, but instead of the homeopathic drug Alcohol 200 (Alc 200, the "vehicle" of the homeopathic drugs) was given to mice that served as positive controls. The observers were blinded during observation as to whether they were looking at a drug-fed or placebo-fed mouse until the codes were deciphered at the end.

Preparation of the potentized homeopathic drugs

Ch 200 and Car 200, made in 90% ethyl alcohol by following the homeopathic principle of dilution and succussion,[4,7] were procured from HAPCO (Kolkata, India). Ch-200 was derived from plant extract of *Chelidonium majus* L. (Papaveraceae). Car-200 was derived from a nosode[8] (confirmed carcinoma of liver).

1 mL each of Ch 200, Car 200, and Alc 200 (potentized ethyl alcohol) was diluted separately with 20 mL of double-distilled water to make the stock solution of Ch 200, Car 200, and Alc 200, respectively. One (1) drop (0.06 mL) of the diluted stock solution of the drugs or alcohol, as appropriate, was orally administered to mice through a specially made pipette.

Laboratory methodology

Slides for study of chromosomal aberrations (CA) were prepared from bone marrow cells by a conventional flame-drying technique.[4,5,9–12] Five hundred (500) cells were examined in each series.

For micronucleus (MN) preparation, smeared bone marrow cells were stained with May-Grunwald stain.[12] Approximately 5000 cells, comprising both polychromatic erythrocytes (PCE) and normochromatic erythrocytes (NCE), were scored.

The mitotic index (MI = dividing/nondividing cells) was assessed from the same slide that was scanned for MN.[13]

For the sperm head anomaly (SHA) study, the technique of Wyrobek et al.[14] was adopted.

The activities of different biomarkers were assessed according to standard methodologies: for lipid peroxidase (LPO),[15] glutamate oxaloacetate (GOT) and glutamate pyruvate transaminase (GPT),[16] and acid phosphatase (ACP) and alkaline phosphatase (ALKP).[17]

Histologic slides of liver at days 60 and 120 were prepared based on standard methods.

For electron microscopy of liver at days 60 and 120, the standard gold coating technique using critical point-drier (CPD-Biorad, Microscience Division, Warford, England), sputter-coater (model 198, Agar Sputter Coater, Stansted, United Kingdom) was adopted in case of scanning electron microscopy (LEO, 435VP, United Kingdom). For transmission electron microscopy (TEM; CM-10, Philips Electron

Table 1. Number of Mice with Tumor Incidence at Different Fixation Intervals and in Different Series

Diet	*# of specimens*	*7 Days*	*15 Days*	*30 Days*	*60 Days*	*90 Days*	*120 Days*
Normal	45	0/5	0/5	0/5	0/10	0/10	0/10
p-DAB+PB	45	0/5	0/5	0/5	10/10	10/10	10/10
p-DAB+PB+Ch 200	45	0/5	0/5	0/5	5/10	4/10	4/10
p-DAB+PB+Car 200	45	0/5	0/5	0/5	5/10	6/10	6/10
p-DAB+PB+Ch 200+Car 200	45	0/5	0/5	0/5	5/10	4/10	4/10
p-DAB+PB+Alc	45	0/5	0/5	1/5	10/10	10/10	10/10

For longer the fixation intervals of 60, 90, and 120 days, 10 mice were used per set, and for shorter fixation intervals of 7, 15, and 30 days five mice were used per set.

Alc, alcohol; Car 200, Carcinosin 200; Ch 200, chelidonium 200; PB, phenobarbital; p-DAB, p-dimethylaminoazobenzene.

Optics, Eindhoven, The Netherlands) the ultra-thin sections (60–90 nm, cut by Reichert E Jung, England) were stained with uranyl acetate and lead citrate. Generally four serial liver sections obtained from each of four different mice at each fixation interval were analyzed.

For analysis of statistical significances the Student's *t* test between different series at fixed time intervals (D+P+Alc versus D+P+Ch 200, D+P+Car 200, and D+P+Ch 200+Car 200), followed by the Kruskal-Wallis multiple comparison test using SPSS software system (version 10.0; SPSS Inc., Chicago, IL) were followed.

RESULTS

The incidence of tumor nodules in different groups of mice is shown in Table 1. In general, both number of tumor nodules and their size appeared to be slightly reduced; quite a few mice even showed liver without tumor nodules in both the p-DAB+PB+Car 200-fed and p-DAB+PB+Ch 200–fed series, more appreciably in the latter. However there was no further reduction in the number of mice showing tumor nodules in the conjointly fed (p-DAB+PB+Ch 200+Car 200) series. On the other hand all mice in the p-DAB+PB+Alc 200–fed series showed tumor nodules as did those in the p-DAB+PB–fed group. The data on different cytogenetic endpoints such as CA, MN, MI, and SHA are summarized in histograms in Figures 1A–1D, and a few representative photographs of typical ones are provided in Figures 2A–2I) The extent of genotoxicity (CA) was generally suppressed or reduced quantitatively in both the p-DAB+PB+Ch 200–fed and p-DAB+PB+Car 200–fed mice compared to p-DAB+PB–fed mice at some specific fixation intervals, but the suppression or reduction was greater ($p < 0.01$ to 0.001) in scale in the conjointly fed mice at all fixation intervals (Tables 2 and 3). On the other hand the genotoxicity and cytotoxicity appeared to be appreciably enhanced in the p-DAB+PB+Alc–fed mice (Fig. 1A) compared to both normal and D+PB–fed mice ($p <$ 0.01 to 0.001).

Similarly the combined drug–fed series showed fewer of micronuclei than either drug-fed series at all fixation inter-

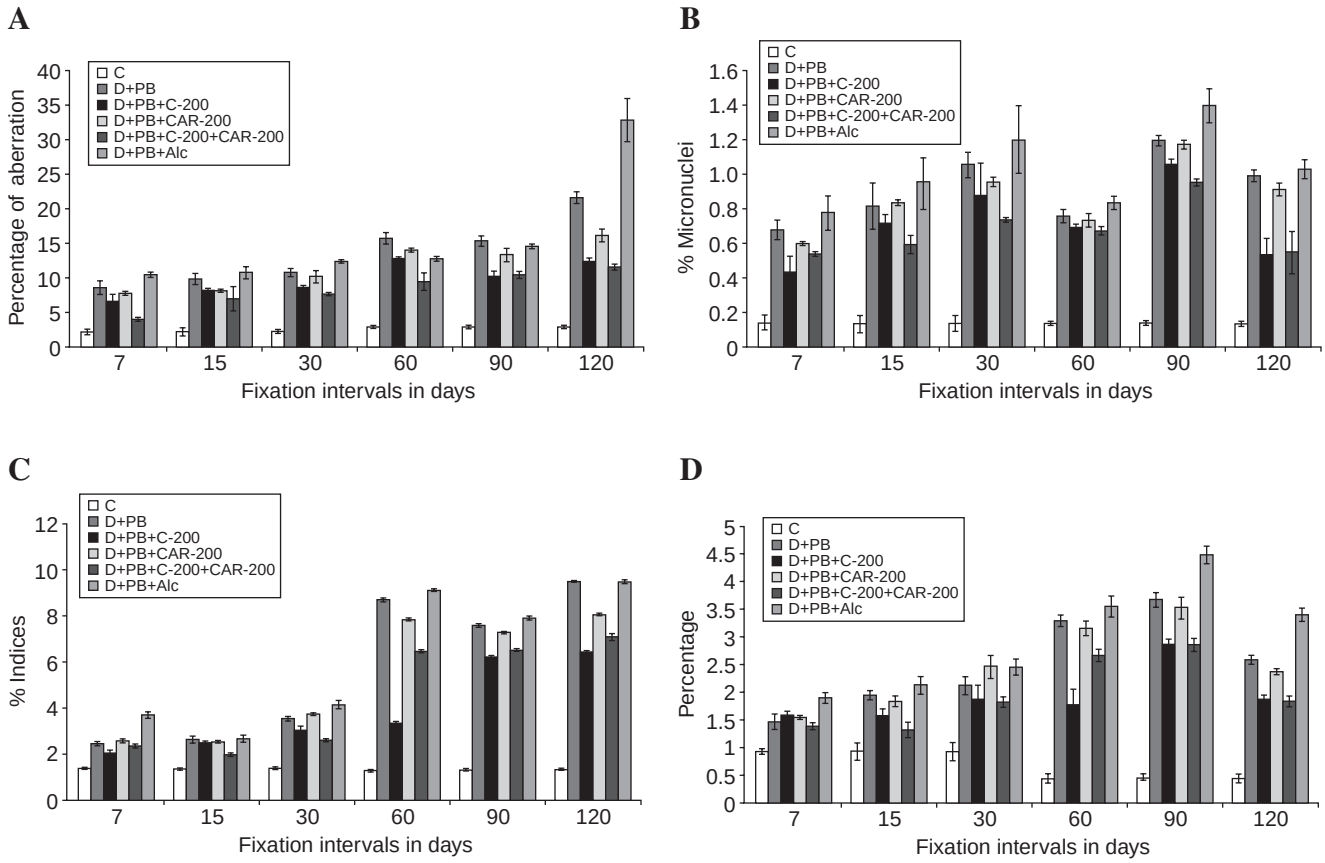

FIG. 1. Histograms showing the following: **A**. percentage of chromosomal alterations (CA) in different series of mice at different fixation intervals; **B**. percentage of micronucleated erythrocytes (MNE) in different series of mice at different fixation intervals; **C**. percentage of mitotic indices in different series of mice at different fixation intervals; and **D**. percentage of sperm head abnormalities in different series of mice at different fixation intervals. Alc, alcohol; CAR, Carcinosin 200; C, Chelidonium 200; D, p-diethylaminoazobenzene; PB, phenobarbital.

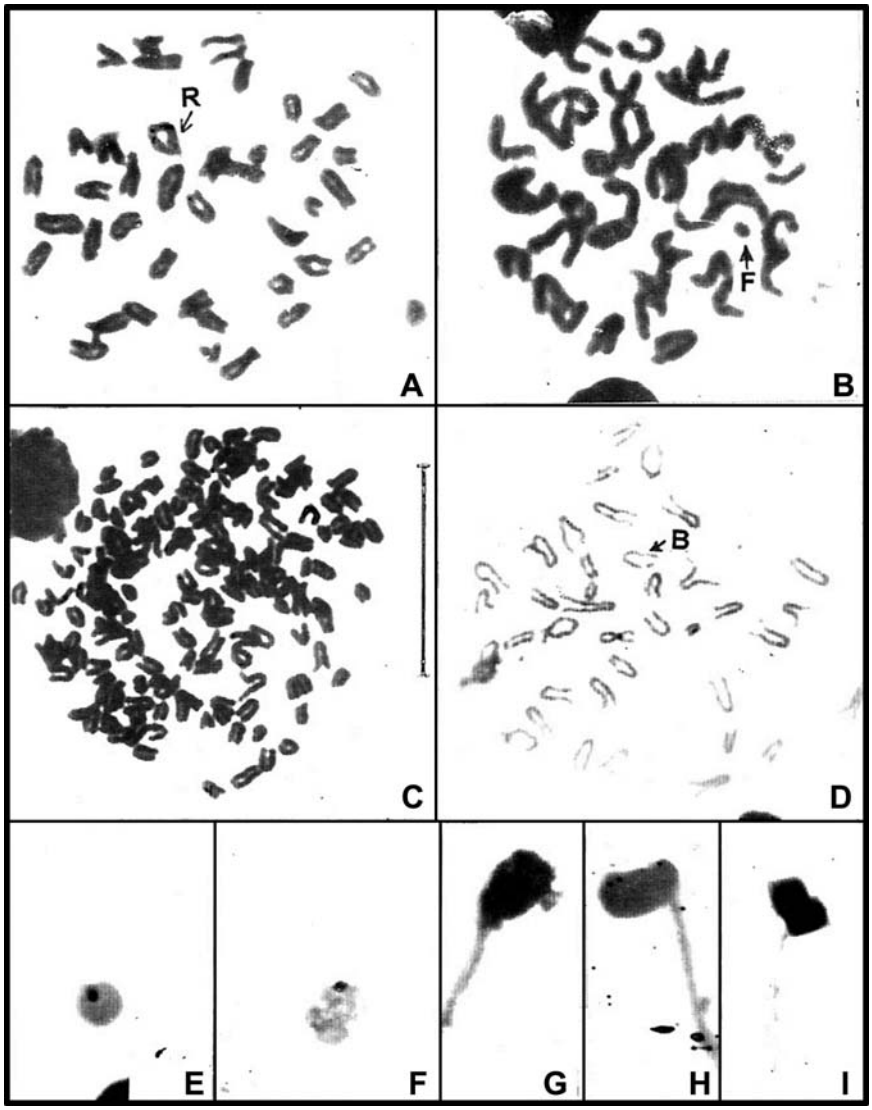

FIG. 2. **A.** Metaphase complement showing ring (R). **B.** Acentric fragment (F with arrow). **C.** Polyploidy. **D.** Break (B with arrow), **E.** Polychromatic erythrocyte showing micronucleus. **F.** Normochromatic erythrocyte showing micronucleus. **G–I.** Sperm with abnormal head morphology. Bar represents 40 μM.

vals ($p < 0.001$), although feeding of drug also brought down the incidence of MN considerably at most fixation intervals (Fig. 1B).

The MI in p-DAB+PB or p-DAB+PB+ Alc 200 steadily increased with time, as compared to values in normal controls (Fig. 1C). However the MI actually decreased in mice fed either Car 200 or Ch 200. The MI was also appreciably reduced in the combined drug–fed series but not to the extent as in the Ch 200–fed mice.

Similarly there was a clear suppression or reduction in the frequency of SHA in mice fed either drug alone or both drugs conjointly, compared to mice given either p-DAB+PB or p-DAB+PB+Alc 200, at most fixation intervals (Fig. 1D).

Data on activities of various toxicity marker enzymes are presented in summarized form in Tables 4–8). There was enhanced lipid peroxidation activity in all three tissues of mice fed with p-DAB+PB and p-DAB+PB+Alc at all fixation intervals compared to normal controls ($p < 0.001$; Table 4). Ch 200 when given along with p-DAB+PB also suppressed or reduced LPO activity in the majority of the tissues and at most fixation intervals, particularly at longer fixation intervals. However the p-DAB+PB+Car 200–fed mice did not show appreciable reduction in LPO activity compared to p-DAB+PB+Ch 200–fed mice. Although the combined feeding of Ch 200 and Car 200 did not show diminished LPO activity at shorter fixation intervals, the mean LPO activity was found to be appreciably reduced ($p < 0.05$ to $p < 0.01$; Table 4) at longer fixation intervals.

Mean activities of GOT appeared to increase remarkably in mice fed with p-DAB+PB and p-DAB+PB+Alc in all

Table 2. Frequency Distribution of Mitotic Index (MI), Chromosome Alterations (CA), Micronucleated Erythrocytes (MN), and Sperm Head Anomaly (SHA) in Mice of Different Experimental and Control Series

Fixation intervals (days)	Series	MI	CA			MN				SHA
		% of MI (% ± SE)	% of Major CA	% of Other CA	Total CA (% ± SE)	% of MN in PCE	% of MN in NCE	P/N	Total MN (% ± SE)	Total SHA (% ± SE)
7	Normal	1.42 ± 0.20	0.4	1.8	2.2 ± 0.55	0.13	0.14	0.81	0.14 ± 0.05	0.94 ± 0.16
	p-DAB+PB	2.50 ± 0.45	1.8	6.8	8.6 ± 0.98	0.73	0.63	0.97	0.68 ± 0.06	1.46 ± 0.16
	p-DAB+PB+Ch 200	2.11 ± 0.68***	1.4	5.2	6.6 ± 1.07**	0.45	0.42	1.09	0.44 ± 0.09***	1.22 ± 0.05**
	p-DAB+PB+Car 200	2.60 ± 0.22***	1.8	6.0	7.8 ± 0.20**	0.61	0.59	0.95	0.60 ± 0.01***	1.56 ± 0.02**
	p-DAB+PB+Ch 200+Car 200	2.40 ± 0.11***	1.0	3.0	4.0 ± 0.32***	0.59	0.49	0.90	0.54 ± 0.02***	1.40 ± 0.07***
	p-DAB+PB+Alc	3.76 ± 0.08	2.4	8.0	10.4 ± 0.51	0.77	0.79	0.96	0.78 ± 0.10	1.92 ± 0.08
15	Normal	1.4 ± 0.20	0.4	1.8	2.20 ± 0.55	0.13	0.14	0.81	0.14 ± 0.05	0.94 ± 0.16
	p-DAB+PB	2.66 ± 0.27	2.0	7.8	9.80 ± 2.08	0.98	0.69	0.75	0.82 ± 0.13	1.96 ± 0.07
	p-DAB+PB+Ch 200	2.52 ± 0.13*	1.4	6.8	8.20 ± 1.66**	0.67	0.68	0.81	0.68 ± 0.04***	1.58 ± 0.13***
	p-DAB+PB+Car 200	2.56 ± 0.27*	1.6	6.6	8.20 ± 0.20**	0.94	0.76	0.74	0.84 ± 0.02*	1.56 ± 0.02***
	p-DAB+PB+Ch 200+Car 200	2.02 ± 0.06***	1.2	5.8	7.0 ± 1.78***	0.72	0.48	0.87	0.60 ± 0.05***	1.40 ± 0.07***
	p-DAB+PB+Alc	2.7 ± 0.27	2.4	8.4	10.80 ± 0.86	1.02	0.91	0.76	0.96 ± 0.74	2.14 ± 0.15
30	Normal	1.4 ± 0.2	0.4	1.8	2.20 ± 0.55	0.13	0.14	0.81	0.14 ± 0.05	0.94 ± 0.16
	p-DAB+PB	3.56 ± 0.21	2.4	8.4	10.80 ± 1.46	0.99	1.12	0.93	1.06 ± 0.07	2.14 ± 0.15
	p-DAB+PB+Ch 200	3.02 ± 0.29***	1.8	6.8	8.60 ± 1.28***	0.85	0.90	0.96	0.88 ± 0.19***	1.88 ± 0.24***
	p-DAB+PB+Car 200	3.76 ± 0.13	2.4	7.8	10.2 ± 0.86***	0.95	0.96	0.85	0.96 ± 0.02***	2.48 ± 0.20
	p-DAB+PB+Ch 200+Car 200	2.62 ± 0.09***	1.2	5.8	7.0 ± 1.78***	0.80	0.68	0.99	0.74 ± 0.02***	1.82 ± 0.11***
	p-DAB+PB+Alc	3.64 ± 0.11	2.6	9.8	12.4 ± 1.63	1.07	1.34	1.09	1.20 ± 0.2	2.46 ± 0.15

*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

Data are expressed as mean ± standard error (SE); values with $p < 0.05$ were considered statistically significant followed by Kruskal-Wallis comparison test.

Alc, alcohol; Car 200, Carcinosin 200; Ch 200, Chelidonium 200; NCE, normochromatic erythrocytes; PB, phenobarbital; PCE, polychromatic erythrocytes; P/N, the ratio of polychromatic to normochromatic erythrocytes.

Table 3. Frequency Distribution of Mitotic Index (MI), Chromosome Alterations (CA), Micronucleated Erythrocytes (ME), and Sperm Head Anomaly (SHA) in Mice of Different Experimental and Control Series

Fixation intervals (days)	*Series*	*MI* (% ± SE)	*CA* % of Major CA	*MN* % of Other CA	*SHA* Total CA (% ± SE)	*% of MN in PCE*	*% of MN in NCE*	*P/N*	*Total MN* (% ± SE)	*Total SHA* (% ± SE)
60	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
	p-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
	p-DAB+PB+Ch 200	3.36 ± 0.37***	4.6	8.2	12.8 ± 3.87	0.45	0.26	0.497	0.32 ± 0.08***	0.81 ± 0.11***
	p-DAB+PB+Car 200	7.84 ± 0.12***	5.0	9.0	9.41 ± 1.36**	0.78	0.69	1.05	0.74 ± 0.04***	3.16 ± 0.14
	p-DAB+PB+Ch 200+Car 200	6.48 ± 0.14***	4.0	5.4	9.00 ± 0.32***	0.49	0.88	1.11	0.68 ± 0.02***	2.68 ± 0.10
	p-DAB+PB+Alc	9.1 ± 0.63	5.6	5.8	11.4 ± 2.01	0.72	0.94	0.97	0.84 ± 0.129	2.72 ± 0.28
90	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
	p-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
	p-DAB+PB+Ch 200	2.90 ± 0.34***	4.6	5.6	10.2 ± 1.56***	0.29	0.48	1.16	0.38 ± 0.11***	1.66 ± 0.16***
	p-DAB+PB+Car 200	7.30 ± 0.15***	5.8	7.8	13.4 ± 0.93	1.26	1.11	0.91	1.18 ± 0.02*	3.54 ± 0.18
	p-DAB+PB+Ch 200+Car 200	6.52 ± 0.06***	3.0	7.4	10.4 ± 0.60***	0.87	1.07	0.93	0.96 ± 0.02***	2.86 ± 0.12***
	p-DAB+PB+Alc	8.12 ± 0.76	7.0	5.0	12.0 ± 0.84	1.73	1.04	0.86	1.36 ± 0.16	3.10 ± 0.11
120	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
	p-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
	p-DAB+PB+Ch 200	6.44 ± 0.35***	3.8	4.2	8.0 ± 0.32***	0.12	0.19	2.18	0.14 ± 0.06***	0.38 ± 0.07***
	p-DAB+PB+Car 200	8.08 ± 0.12**	7.6	8.6	16.2 ± 0.92***	0.75	1.09	1.11	0.92 ± 0.04***	2.38 ± 0.05***
	p-DAB+PB+Ch 200+Car 200	7.10 ± 0.12***	4.4	7.2	11.6 ± 0.51***	0.55	0.56	0.88	0.56 ± 0.10***	1.84 ± 0.11***
	p-DAB+PB+Alc	9.86 ± 0.65	15.0	17.8	32.8 ± 1.35	1.64	1.34	0.82	1.48 ± 0.15	3.71 ± 0.07

*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

Data are expressed as mean ± standard error (SE); values with $p < 0.05$ were considered statistically significant followed by Kruskal-Wallis comparison test.

Al, alcohol; Car 200, Carcinosin 200; Ch200, Chelidoniium 200; NCE, normochromatic erythrocytes; PB = phenobarbital; PCE, polychromatic erythrocytes.

Table 4. Mean Lipid Peroxidase (LPO) Activity in (nmol/L MDA/g tissue) in Different Tissues of Control and Treated Mice at Different Fixation Intervals

Fixation intervals in days Series	*7 Days LPO activity (activity ± SE)*			*15 Days LPO activity (activity ± SE)*			*30 Days LPO activity (activity ± SE)*		
	Liver	*Kidney*	*Spleen*	*Liver*	*Kidney*	*Spleen*	*Liver*	*Kidney*	*Spleen*
Normal	9.08 ± 0.55	6.10 ± 0.88	10.13 ± 1.03	7.79 ± 1.51	5.38 ± 0.71	9.48 ± 0.88	6.92 ± 1.03	7.38 ± 0.92	9.38 ± 0.66
p-DAB+PB	32.25 ± 1.78	39.51 ± 0.88	36.20 ± 1.07	57.66 ± 0.56	34.52 ± 4.17	33.84 ± 1.80	87.89 ± 2.98	54.41 ± 1.01	68.15 ± 0.89
p-DAB+PB+ Ch 200	54.23 ± 2.25***	31.84 ± 2.42*	28.68 ± 4.44***	21.42 ± 8.06**	10.15 ± 1.12***	39.17 ± 7.49	39.99 ± 0.54***	45.99 ± 1.17***	91.79 ± 0.76
p-DAB+PB+ Car 200	56.66 ± 5.62**	38.59 ± 3.47	28.27 ± 3.84***	39.02 ± 0.17***	22.97 ± 1.01***	28.89 ± 1.29***	66.05 ± 2.75***	53.02 ± 0.79	78.10 ± 0.48
p-DAB+PB+ Ch 200+ Car 200	39.66 ± 6.37	34.06 ± 2.11*	29.36 ± 4.15***	20.14 ± 3.18***	41.78 ± 6.42	31.53 ± 0.24***	45.17 ± 0.73***	40.10 ± 0.76***	49.63 ± 1.28***
p-DAB+PB+ Alc	43.05 ± 0.94	47.06 ± 0.55	41.36 ± 1.02	69.67 ± 0.18	31.53 ± 0.66	42.92 ± 6.41	69.02 ± 1.65	60.61 ± 1.38	90.02 ± 2.94
	60 Days			*90 Days*			*120 Days*		
Normal	9.55 ± 0.21	7.94 ± 1.16	10.92 ± 1.29	9.07 ± 0.42	9.74 ± 1.20	10.76 ± 1.77	9.93 ± 0.84	10.15 ± 1.20	12.17 ± 1.03
p-DAB+PB	101.16 ± 11.55	70.31 ± 2.19	74.51 ± 1.33	67.82 ± 5.11	45.17 ± 1.74	71.02 ± 0.73	97.54 ± 0.41	92.41 ± 0.70	63.79 ± 0.53
p-DAB+PB+ Ch 200	67.48 ± 1.26*	50.10 ± 1.64***	67.74 ± 2.75**	43.69 ± 4.33*	33.94 ± 4.18**	34.51 ± 1.11***	56.15 ± 0.34***	34.92 ± 1.25***	46.51 ± 0.17***
p-DAB+PB+ Car 200	74.31 ± 1.29***	54.61 ± 0.91***	66.73 ± 2.04**	47.64 ± 3.28*	33.12 ± 1.61**	47.74 ± 0.43***	94.87 ± 0.51	47.43 ± 0.81***	19.94 ± 0.25***
p-DAB+PB+ Ch 200+ Car 200	61.95 ± 2.73**	48.45 ± 2.28***	58.33 ± 3.72**	35.64 ± 6.30***	24.87 ± 2.87***	33.74 ± 1.29***	61.54 ± 7.08***	28.37 ± 2.11***	42.92 ± 1.13**
p-DAB+PB+ Alc	82.31 ± 1.85	78.20 ± 0.69	90.1 ± 2.11	94.65 ± 5.68	51.89 ± 3.25	78.20 ± 0.42	69.28 ± 0.75	68.92 ± 1.43	43.58 ± 0.79

*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.
Statistical analysis done between series p-DAB+PB+Alc versus p-DAB+PB+Ch 200, p-DAB+PB+Car 200, p-DAB+PB+Ch 200+Car 200.
Alc, alcohol; Car 200, Carcinocen 200; Ch 200, Chelidonium 200; PB, phenobarbital; SE, standard error.

Table 5. Mean Glutamate Oxaloacetate Transaminase (GOT) Activities in (mmol/L per min per mg Protein) in Different Tissues of Control and Treated Mice at Different Fixation Intervals

	7 Days GOT activity (activity ± SE)			*15 Days GOT activity (activity ± SE)*			*30 Days GOT (activity ± SE)*		
Series	*Liver*	*Kidney*	*Spleen*	*Liver*	*Kidney*	*Spleen*	*Liver*	*Kidney*	*Spleen*
Normal	0.08 ± 0.01	0.16 ± 0.02	0.06 ± 0.01	0.08 ± 0.01	0.11 ± 0.03	0.05 ± 0.01	0.05 ± 0.01	0.04 ± 0.01	0.04 ± 0.004
p-DAB+PB	0.53 ± 0.01	0.41 ± 0.06	0.44 ± 0.01	0.69 ± 0.01	0.97 ± 0.01	0.44 ± 0.13	1.20 ± 0.01	3.21 ± 0.02	0.41 ± 0.04
p-DAB+PB+Ch 200	0.45 ± 0.02	0.70 ± 0.004	0.26 ± 0.01***	0.36 ± 0.01***	0.83 ± 0.02	0.39 ± 0.08*	0.69 ± 0.13***	1.25 ± 0.01***	0.17 ± 0.002***
p-DAB+PB+Car 200	0.84 ± 0.09	0.93 ± 0.01	0.37 ± 0.01*	0.55 ± 0.01*	0.38 ± 0.03***	0.36 ± 0.02*	1.07 ± 0.16*	0.82 ± 0.04***	0.20 ± 0.003***
p-DAB+PB+Ch 200+ Car 200	0.53 ± 0.01	0.24 ± 0.01**	0.17 ± 0.01***	0.45 ± 0.01**	0.61 ± 0.01**	0.29 ± 0.01***	1.06 ± 0.01*	1.07 ± 0.08***	0.34 ± 0.01
p-DAB+PB+Alc	0.53 ± 0.01	0.38 ± 0.01	0.30 ± 0.004	0.78 ± 0.01	1.24 ± 0.01	0.45 ± 0.01	1.28 ± 0.003	1.07 ± 0.01	0.35 ± 0.003
	60 Days			*90 Days*			*120 Days*		
Normal	0.07 ± 0.01	0.06 ± 0.03	0.06 ± 0.01	0.07 ± 0.01	0.06 ± 0.01	0.11 ± 0.03	0.05 ± 0.004	0.05 ± 0.01	0.03 ± 0.003
p-DAB+PB	0.54 ± 0.01	1.18 ± 0.01	0.19 ± 0.004	0.77 ± 0.01	0.88 ± 0.02	0.41 ± 0.03	0.87 ± 0.001	0.79 ± 0.001	0.33 ± 0.002
p-DAB+PB+Ch 200	1.09 ± 0.02	0.55 ± 0.001***	0.21 ± 0.004	0.97 ± 0.01	0.64 ± 0.01	0.34 ± 0.02*	0.79 ± 0.002*	1.22 ± 0.004	0.23 ± 0.001*
p-DAB+PB+Car 200	0.42 ± 0.003***	1.07 ± 0.01**	0.25 ± 0.01	0.95 ± 0.01	0.93 ± 0.01	0.36 ± 0.04*	0.59 ± 0.002***	0.73 ± 0.01	0.31 ± 0.001
p-DAB+PB+Ch 200+ Car 200	0.55 ± 0.02	0.68 ± 0.01***	0.19 ± 0.003	0.63 ± 0.01	0.82 ± 0.01	0.25 ± 0.01***	0.76 ± 0.004*	1.03 ± 0.01	0.44 ± 0.002
p-DAB+PB+Alc	0.79 ± 0.03	1.06 ± 0.01	0.22 ± 0.003	0.67 ± 0.02	0.99 ± 0.03	0.54 ± 0.06	0.76 ± 0.003	0.68 ± 0.003	0.43 ± 0.004

*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

Statistical analysis done between series p-DAB+PB+Alc versus p-DAB+PB+Ch 200, p-DAB+PB+Car 200, p-DAB+PB+Ch 200+Car 200.

Alc, alcohol; Car 200, Carcinocen 200; Ch 200, Chelidonium 200; PB, phenobarbital; SE, standard error.

Table 6. Mean Glutamate Pyruvate Transaminase (GPT) Activities in (mmol/L per min per mg Protein) in Different Tissues of Control and Treated Mice at Different Fixation Intervals

	7 Days GPT activity (activity ± SE)			*15 Days GPT activity (activity ± SE)*			*30 Days GPT activity (activity ± SE)*		
Series	*Liver*	*Kidney*	*Spleen*	*Liver*	*Kidney*	*Spleen*	*Liver*	*Kidney*	*Spleen*
Normal	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.03	0.03 ± 0.01	0.03 ± 0.004	0.04 ± 0.004	0.04 ± 0.01	0.04 ± 0.003	0.04 ± 0.01
p-DAB+PB	0.53 ± 0.01	0.41 ± 0.06	0.44 ± 0.01	0.48 ± 0.002	0.19 ± 0.004	0.09 ± 0.004	0.81 ± 0.01	0.42 ± 0.01	0.15 ± 0.003
p-DAB+PB+Ch 200	0.84 ± 0.09	0.93 ± 0.01	0.36 ± 0.01***	0.38 ± 0.02*	0.16 ± 0.01	0.08 ± 0.003	0.70 ± 0.01**	0.12 ± 0.003***	0.05 ± 0.002***
p-DAB+PB+Car 200	0.84 ± 0.09	0.93 ± 0.01	0.36 ± 0.01*	0.41 ± 0.01*	0.07 ± 0.01**	0.07 ± 0.002	0.87 ± 0.03	0.12 ± 0.003***	0.04 ± 0.001***
p-DAB+PB+Ch 200+ Car 200	0.53 ± 0.01	0.24 ± 0.01***	0.17 ± 0.01***	0.35 ± 0.01**	0.11 ± 0.001***	0.05 ± 0.01	0.63 ± 0.02***	0.21 ± 0.004*	0.07 ± 0.003***
p-DAB+PB+Alc	0.53 ± 0.01	0.38 ± 0.01	0.30 ± 0.004	0.78 ± 0.01	1.24 ± 0.01	0.45 ± 0.01	0.84 ± 0.01	0.16 ± 0.002	0.15 ± 0.14
	60 Days			*90 Days*			*120 Days*		
Normal	0.06 ± 0.01	0.04 ± 0.01	0.04 ± 0.01	0.04 ± 0.004	0.03 ± 0.001	0.04 ± 0.005	0.03 ± 0.01	0.04 ± 0.01	0.03 ± 0.004
p-DAB+PB	0.55 ± 0.04	0.19 ± 0.01	0.06 ± 0.002	0.61 ± 0.01	0.19 ± 0.01	0.12 ± 0.003	0.69 ± 0.01	0.18 ± 0.004	0.11 ± 0.003
p-DAB+PB+Ch 200	0.69 ± 0.01	0.08 ± 0.01***	0.06 ± 0.002	0.79 ± 0.003	0.13 ± 0.01	0.10 ± 0.003	0.63 ± 0.01*	0.27 ± 0.01	0.07 ± 0.001***
p-DAB+PB+Car 200	0.28 ± 0.04***	0.19 ± 0.01	0.08 ± 0.01	0.76 ± 0.01	0.20 ± 0.005	0.10 ± 0.004	0.47 ± 0.001***	0.16 ± 0.003	0.10 ± 0.001
p-DAB+PB+Ch 200+ Car 200	0.39 ± 0.01	0.11 ± 0.003*	0.05 ± 0.004	0.49 ± 0.01	0.15 ± 0.03*	0.07 ± 0.002***	0.62 ± 0.01*	0.21 ± 0.002	0.12 ± 0.002
p-DAB+PB+Alc	0.55 ± 0.01	0.19 ± 0.01	0.08 ± 0.01	0.76 ± 0.01	0.22 ± 0.02	0.17 ± 0.01	0.61 ± 0.002	0.16 ± 0.002	0.15 ± 0.004

*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.
Statistical analysis done between series p-DAB+PB+Alc versus p-DAB+PB+Ch 200, p-DAB+PB+Car 200, p-DAB+PB+Ch 200+Car 200.
Alc, alcohol; Car 200, Carcinocen 200; Ch 200, Chelidonium 200; PB, phenobarbital; SE, standard error.

Table 7. Mean Acid Phosphatase (ACP) Activities (mmol/L Phenol Liberated/100 mg Protein) in Different Tissues of Control and Treated Mice at Different Fixation Intervals

	7 Days ACP activity (activity ± SE)			*15 Days ACP activity (activity ± SE)*			*30 Days ACP (activity ± SE)*		
Series	*Liver*	*Kidney*	*Spleen*	*Liver*	*Kidney*	*Spleen*	*Liver*	*Kidney*	*Spleen*
Normal	0.04 ± 0.01	0.08 ± 0.004	0.10 ± 0.01	0.09 ± 0.04	0.09 ± 0.01	0.09 ± 0.01	0.08 ± 0.01	0.08 ± 0.01	0.09 ± 0.01
p-DAB+PB	0.74 ± 0.02	0.76 ± 0.03	0.86 ± 0.001	0.73 ± 0.02	0.78 ± 0.01	0.78 ± 0.01	0.85 ± 0.001	0.72 ± 0.002	0.78 ± 0.004
p-DAB+PB+Ch 200	0.64 ± 0.01***	0.29 ± 0.01***	0.54 ± 0.1***	0.47 ± 0.004***	0.56 ± 0.002***	0.67 ± 0.01***	0.67 ± 0.03***	0.39 ± 0.002*	0.50 ± 0.004***
p-DAB+PB+Car 200	0.62 ± 0.01***	0.52 ± 0.01***	0.79 ± 0.01***	0.59 ± 0.01***	0.55 ± 0.02***	0.70 ± 0.004***	0.56 ± 0.003*	0.51 ± 0.002***	0.73 ± 0.001
p-DAB+PB+Ch 200+ Car 200	0.31 ± 0.03***	0.27 ± 0.00***	0.60 ± 0.02***	0.39 ± 0.01***	0.47 ± 0.01***	0.63 ± 0.01***	0.49 ± 0.004***	0.22 ± 0.002***	1.25 ± 0.06
p-DAB+PB+Alc	0.74 ± 0.01	0.77 ± 0.01	0.85 ± 0.003	0.79 ± 0.02	0.79 ± 0.001	0.82 ± 0.002	0.74 ± 0.01	0.98 ± 0.01	0.93 ± 0.01
	60 Days			*90 Days*			*120 Days*		
Normal	0.06 ± 0.01	0.08 ± 0.01	0.11 ± 0.01	0.09 ± 0.01	0.95 ± 0.003	0.10 ± 0.01	0.11 ± 0.01	0.09 ± 0.01	0.11 ± 0.01
p-DAB+PB	0.82 ± 0.01	1.05 ± 0.01	1.14 ± 0.01	0.68 ± 0.01	0.82 ± 0.03	1.14 ± 0.02	1.05 ± 0.002	0.71 ± 0.001	1.01 ± 0.001
p-DAB+PB+Ch 200	1.08 ± 0.1	0.56 ± 0.01***	0.60 ± 0.01***	0.59 ± 0.02***	0.57 ± 0.02***	0.56 ± 0.04***	0.75 ± 0.01***	0.84 ± 0.001	0.48 ± 0.00***
p-DAB+PB+Car 200	0.59 ± 0.001***	0.98 ± 0.02***	0.59 ± 0.01***	0.91 ± 0.02	0.33 ± 0.01***	0.69 ± 0.02*	1.18 ± 0.001	1.33 ± 0.001	1.26 ± 0.002
p-DAB+PB+Ch 200+ Car 200	0.83 ± 0.01	0.63 ± 0.01***	0.61 ± 0.01***	0.93 ± 0.21	0.78 ± 0.06***	0.80 ± 0.01*	0.68 ± 0.001	0.64 ± 0.003	1.98 ± 0.001
p-DAB+PB+Alc	1.02 ± 0.01	1.09 ± 0.04	1.44 ± 0.02	1.44 ± 0.13	0.99 ± 0.04	1.58 ± 0.02	0.69 ± 0.002	0.64 ± 0.01	0.75 ± 0.01

*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

Statistical analysis done between series p-DAB+PB+Alc versus p-DAB+PB+Ch 200, p-DAB+PB+Car 200, p-DAB+PB+Ch 200+Car 200.

Alc, alcohol; Car 200, Carcinosin 200; Ch 200, Chelidonium 200; PB, phenobarbital; SE, standard error.

Table 8. Mean Alkaline Phosphatase (ALKP) Activities in (mmol/L Phenol Liberated/100 mg Protein) in Different Tissues of Control and Treated Mice at Different Fixation Intervals

	7 Days ALKP activity (activity ± SE)			*15 Days ALKP activity (activity ± SE)*			*30 Days ALKP (activity ± SE)*		
Series	*Liver*	*Kidney*	*Spleen*	*Liver*	*Kidney*	*Spleen*	*Liver*	*Kidney*	*Spleen*
Normal	0.06 ± 0.01	0.07 ± 0.004	0.09 ± 0.01	0.89 ± 0.01	0.08 ± 0.01	0.09 ± 0.01	0.06 ± 0.01	0.07 ± 0.01	0.09 ± 0.01
p-DAB+PB	0.59 ± 0.01	1.75 ± 0.02	0.14 ± 0.01	0.57 ± 0.01	1.73 ± 0.04	0.15 ± 0.004	0.94 ± 0.07	1.12 ± 0.03	1.13 ± 0.01
p-DAB+PB+Ch 200	0.02 ± 0.001***	0.40 ± 0.001***	0.02 ± 0.002***	0.51 ± 0.01	1.49 ± 0.02***	0.13 ± 0.01	0.23 ± 0.004***	0.38 ± 0.02***	1.30 ± 0.01
p-DAB+PB+Car 200	0.04 ± 0.01***	0.68 ± 0.04***	0.48 ± 0.001	0.56 ± 0.01	1.15 ± 0.05***	0.15 ± 0.01	0.52 ± 0.01***	0.40 ± 0.01***	1.12 ± 0.01
p-DAB+PB+Ch 200+ Car 200	0.20 ± 0.00***	0.48 ± 0.01***	0.03 ± 0.002***	0.54 ± 0.01	0.97 ± 0.01***	0.12 ± 0.001	0.42 ± 0.04***	0.40 ± 0.04***	0.58 ± 0.02***
p-DAB+PB+Alc	0.59 ± 0.01	1.71 ± 0.12	0.14 ± 0.004	0.61 ± 0.01	1.83 ± 0.01	0.16 ± 0.01	0.98 ± 0.22	1.28 ± 0.01	1.57 ± 0.01
	60 Days			*90 Days*			*120 Days*		
Normal	0.05 ± 0.03	0.06 ± 0.002	0.07 ± 0.01	0.05 ± 0.01	0.05 ± 0.004	0.09 ± 0.01	0.04 ± 0.002	0.04 ± 0.004	0.07 ± 0.01
p-DAB+PB	0.49 ± 0.02	0.54 ± 0.01	1.14 ± 0.04	0.71 ± 0.01	0.67 ± 0.02	0.37 ± 0.01	0.76 ± 0.001	0.83 ± 0.00	0.29 ± 0.00
p-DAB+PB+Ch 200	0.25 ± 0.01***	0.57 ± 0.04	1.06 ± 0.02	0.39 ± 0.02***	0.99 ± 0.04	0.30 ± 0.002	0.32 ± 0.002***	0.61 ± 0.01**	0.31 ± 0.00
p-DAB+PB+Car 200	0.35 ± 0.01***	0.48 ± 0.03*	1.11 ± 0.04	0.41 ± 0.03***	0.58 ± 0.01**	0.19 ± 0.03***	0.36 ± 0.001***	0.52 ± 0.01***	1.41 ± 0.003
p-DAB+PB+Ch 200+ Car 200	0.17 ± 0.003***	0.17 ± 0.001***	1.04 ± 0.01**	0.27 ± 0.02***	0.26 ± 0.001***	0.22 ± 0.01***	0.27 ± 0.02***	0.22 ± 0.01***	0.31 ± 0.001
p-DAB+PB+Alc	0.71 ± 0.03	0.48 ± 0.01	1.29 ± 0.07	0.75 ± 0.02	0.92 ± 0.001	0.41 ± 0.01	0.90 ± 0.002	0.73 ± 0.03	0.26 ± 0.001

*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

Statistical analysis done between series p-DAB+PB+Alc versus p-DAB+PB+Ch 200, p-DAB+PB+Car 200, p-DAB+PB+Ch 200+Car 200.

Alc, alcohol; Car 200, Carcinocen 200; Ch 200, Chelidonium 200; PB, phenobarbital; SE, standard error.

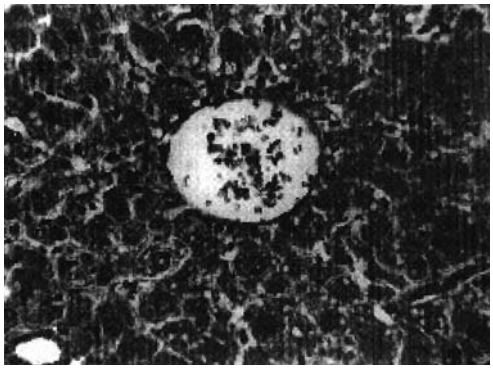

FIG. 3. Histologic section of liver of mouse fed p-diethylaminoazobenzene and phenobarbital (p-DAB+PB).

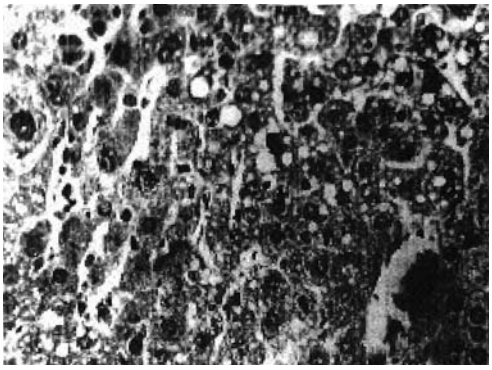

FIG. 5. Section of liver of mouse fed p-diethylaminoazobenzene, phenobarbital, and Carcinosin 200 (p-DAB+PB+Car-200).

tissues and at all fixation intervals compared to values in normal controls ($p < 0.001$). The p-DAB+PB+Ch 200–fed mice generally showed a trend of decrease, sometimes marginally and sometimes quite appreciably in certain tissues and at certain fixation intervals. However a more pronounced reduction in GOT activity was found in some tissue of conjointly drug-fed mice at some specific fixation intervals ($p < 0.05$ to $p < 0.001$; Table 5).

There was an increase in GPT activity in mice fed p-DAB+PB and p-DAB+PB+Alc. In the *p*-DAB+PB+Car 200–fed series the GPT activity appeared to be somewhat erratic, slightly decreased or even sometimes slightly increased in some tissues, at some fixation intervals (Table 6). However in the p-DAB+PB+Ch 200–fed mice the decrease in GPT activity was appreciable at most fixation intervals, and was the case in the combined drug–fed series.

Compared to controls, there was an increase in the ACP activity in the mice given p-DAB+PB and p-DAB+PB+Alc ($p < 0.001$), but the activity substantially decreased in those fed p-DAB+PB+Ch-200 and combined drugs ($p < 0.05$ to $p < 0.001$), although not consistently at every fixation interval (Table 7).

Similarly, there was an increase in the ALKP activity in mice given p-DAB+PB and p-DAB+PB+Alc ($p < 0.001$), while the activity was substantially decreased in both the p-DAB+PB+Ch 200–fed and combined drug–fed series (Table 8), compared to the p-DAB+PB and the p-DAB+PB+Alc–fed series.

Overall the mice that were fed either drug, alone or in combination, showed effective modulations of their enzyme levels indicative of the protective nature of the drugs, which were slightly more pronounced when the drugs were conjointly given.

Under optical microscopy, the liver sections of p-DAB+PB–fed mice at day 120 (Fig. 3) revealed more drastic tissue damage and necrosis than at day 60 compared to the liver sections of normal controls. A few notable areas of damage caused by the carcinogens were as follows: more than one nucleus was present in some hepatocytes, excessive fibrosis in the hepatic parenchyma was noticed; and cytoplasmic boundaries of the hepatic cells were barely recognizable and also more infiltration of leukocytes among the parenchyma was evident. In the p-DAB+PB+Ch 200–fed series (Fig. 4) the overall effect was considerably

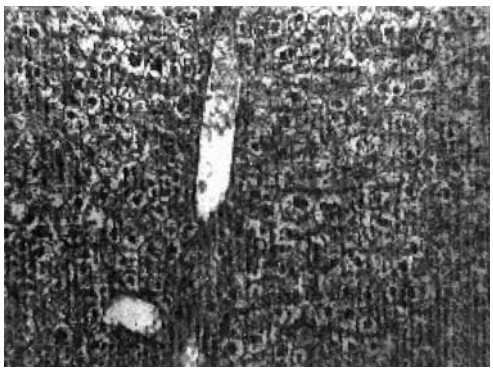

FIG. 4. Section of liver of mouse fed p-diethylaminoazobenzene, phenobarbital, and Chelidonium 200 (p-DAB+PB+Ch-200).

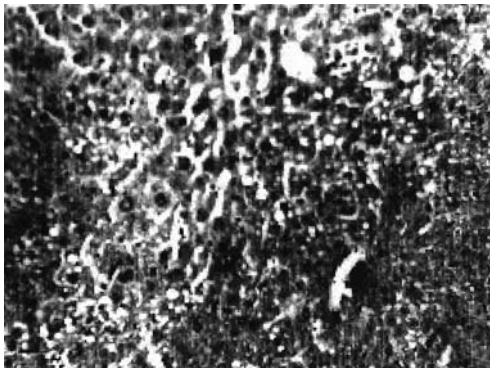

FIG. 6. Liver of mouse fed p-diethylaminoazobenzene, phenobarbital, Chelidonium 200, and Carcinosin 200 (p-DAB+PB+Ch-200+Car-200).

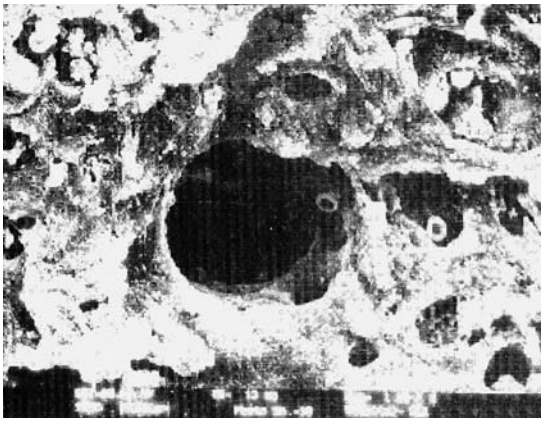

FIG. 7. Section of liver under scanning electron microscopy from mouse fed p-diethylaminoazobenzene and phenobarbital (p-DAB+PB) (magnification 1.02 ×1000).

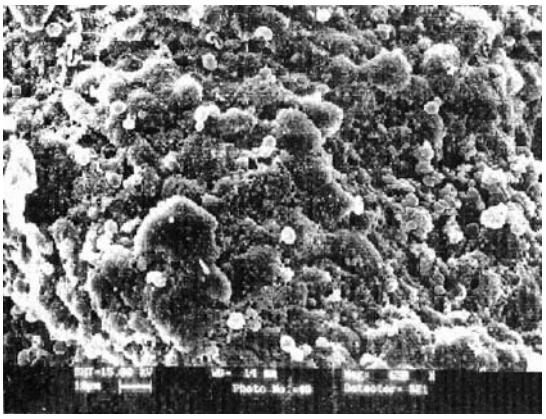

FIG. 9. Liver sections of mice fed p-diethylaminoazobenzene, phenobarbital, and Carcinosin 200 (p-DAB+PB+Car-200) (magnification ×650).

reduced (e.g., the binucleate or multinucleate cells were much fewer, and more cells had distinct boundaries. In the p-DAB+PB+Car 200–fed series (Fig. 5), although the number of unhealthy Kupffer cells was not drastically reduced, infiltration of the cells in the parenchyma was not evident. However in the combined drug–fed series (Fig. 6), relatively less loss of cytoplasm was evident; the nuclei of the cells were found to be intact; and vacuolated cells were fewer.

Analysis with scanning electron microscopy (SEM) revealed that fibrosis was evident; there was tissue necrosis causing the appearance of holes; and a blood–liver barrier was not present in the p-DAB+PB–fed series (Fig. 7). Furthermore, red blood cells (RBCs) were found among parenchymal tissue, which was suggestive of breakdown of the blood–liver barrier. In the p-DAB+PB+Ch 200–fed series (Fig. 8), most cells had intact nuclei and some of the cells were arranged in chords; but still the blood liver barrier did not appear to be intact as many RBCs were located outside the cells. In the p-DAB+PB+Car 200–fed series (Fig. 9) tissue damage was evident but few of the Kuffer cells were present and the blood–liver barrier was broken. In the combined drug–fed series (Fig. 10), edematous swelling was not evident and tissue necrosis was less persistent; a few cells, apparently newly generated, were found to be arranged in chords.

Examination with TEM also revealed damage to intercellular organelles in the p-DAB+PB–fed series at both days 60 and 120 (Fig. 11). A few notable changes were as follows: the endoplasmic reticulum was broken and numerous mitochondria were present in each cell; Kupffer cells were activated; and lipid droplets were numerous. The activation of Kupffer cells might suggest increased secretion of lymphokines. In p-DAB+PB+Ch 200–fed mice (Fig. 12), lipid droplets were few and the endoplasmic reticulum was less broken. The number of Kupffer cells was also fewer. In p-DAB+PB+Car 200–fed mice (Fig. 13); however, the recovery or protection was not as convincing and striking as

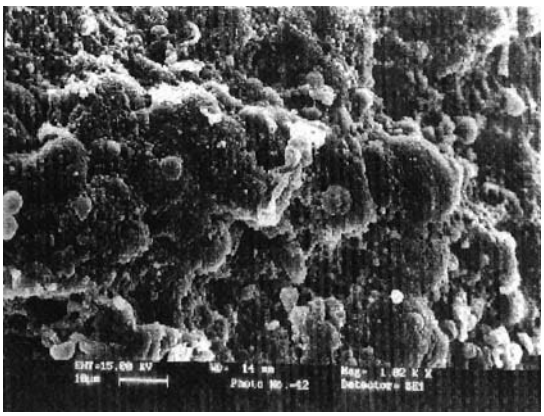

FIG. 8. Section of liver of mouse fed p-diethylaminoazobenzene, phenobarbital, and Chelidonium 200 (p-DAB+PB+Ch-200) (magnification 1.02 ×1000).

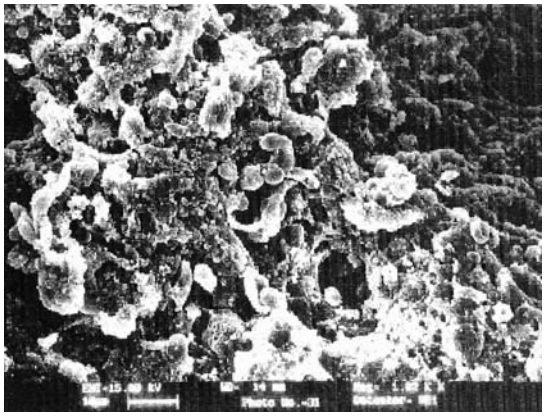

FIG. 10. Liver sections of mice fed p-diethylaminoazobenezene, phenobarbital, Chelidonium 200, and Carcinosin 200 (p-DAB+PB+Ch-200+Car-200) (magnification 1.02 ×1000).

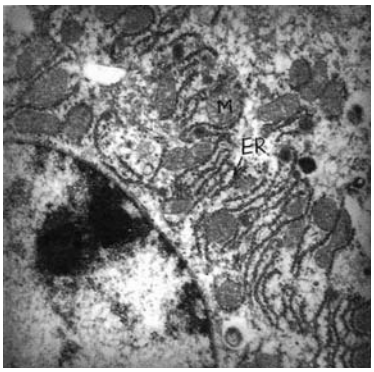

FIG. 11. Sections of liver under transmission electron microscopy from mice fed p-diethylaminoazobenzene and phenobarbital (p-DAB+PB). ER, broken endoplasmic reticulum; M, mitochondria.

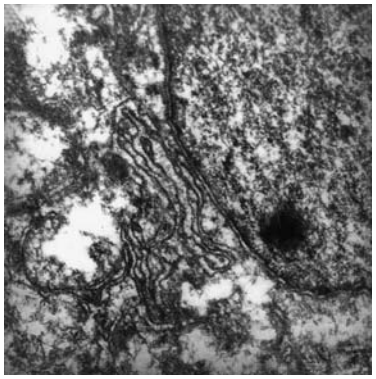

FIG. 13. Liver sections of mice fed p-diethylaminoazobenzene, phenobarbital, and Carcinosin 200 (p-DAB+PB+Car 200).

either in p-DAB+PB+Ch 200–fed or combined drug–fed mice (Fig. 14). In the latter series damage to hepatocytes was not fully recovered, but the numbers of mitochondria, lipid droplets, and broken ERs were fewer.

DISCUSSION

The efficacy of Car 200, given alone or in combination with Ch 200, has not previously been tested. Furthermore, evidence of modulations induced by any potentized homeopathic drug at the ultrastructural level seems not to have been documented earlier. The results of this study demonstrate that Car 200, when administered orally to mice chronically fed p-DAB+PB, apparently showed some amount of antitumoric, anticlastogenic, and anticytotoxic effects; but the efficacy was apparently less than that of the Ch 200–fed group. However the conjoint treatment of Ch 200 and Car 200, which is often adopted by homeopathic practitioners in case of a suspected liver cancer, showed a more pronounced potential of antagonism and protective action against the carcinogens.

Different aspects of how p-DAB and PB induce hepatocarcinogenesis through their degradation and action of their metabolic products have already been extensively studied.[3,18–22] Covalent binding of the metabolites of p-DAB (such as, monoaminoazobenzene and aminoazobenzene) with DNA is believed to be a major carcinogeneic factor.[22] Thus the potentized homeopathic drugs appear to have the ability to block or interfere with the carcinogenic action of carcinogens, either by directly antagonizing their effects or by reversing their ill effects to a considerable extent.

Although both Ch 200 and Car 200 were made in same alcoholic vehicle as Alc 200, only the former showed some protective action against the formation of the liver tumor nodules (few of which at day 60 may actually represent preneoplastic lesions, although others were tumors) whereas the Alc 200 did not.

Crude extracts of the *Chelidonium majus* plant have been reported to have several isoquinoline alkaloids, and both crude extracts and purified compounds derived from *C. majus* have been reported to exhibit antiviral, anti-inflammatory, antitumoric, and antimicrobial properties, as well as inhibitory effects on growth of keratinocytes in human beings[23]

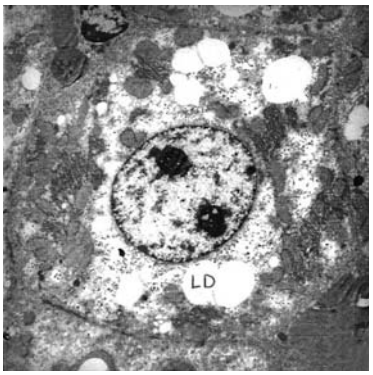

FIG. 12. Liver sections of mice fed p-diethylaminoazobenzene, phenobarbital, and Chelidonium 200 (p-DAB+PB+Ch-200). LD, liquid droplets.

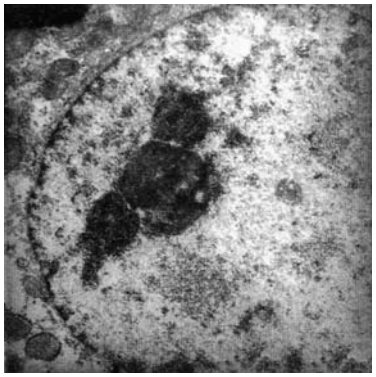

FIG. 14. Liver sections of mice fed p-diethylaminoazobenzene, phenobarbital, Chelidonium 200, and Carcinosin 200 (p-DAB+PB+Ch-200+Car-200). Bar represents 40 μM.

and on lipoxygenase activity in mice.[24] In addition a stimulatory effect has been reported on bile acid–independent flow in the isolated perfused rat liver. However it is difficult to explain how the ultra-high dilutions of Chelidonium could successfully combat such strong carcinogens by bringing about so many positive changes. Because of their minimal side-effects, these homeopathic remedies may serve as potential candidates for future trials in complementary and alternative medicine (CAM) examine their suitability for treatment and management of human liver cancer.

Because the various changes noted in this study can only be brought about ultimately by the activity of certain genes, it may be hypothetized[7,25] that these potentized drugs could somehow manage to correct the expression of certain relevant genes (presumably by eliciting suitable signals to trigger "on" or "off" specific genes), the regulation of which had faltered because of the carcinogens, and for which they had started functioning abnormally.

CONCLUSIONS

As most conventional chemopreventive therapies used at present have toxic side-effects that preclude their effective use in many cases, alternative agents with minimal side-effects on normal cells that effectively destroy or inhibit cancer cells need to be identified. Therefore CAM is gradually becoming popular, particularly in oncology, which often requires a number of therapies, from homeopathy to acupuncture,[26,27] especially as supporting palliative measures.[28–32] Recently a homeopathic drug, Ruta 6, has been reported to induce cell death in brain cancer cells,[35] as revealed by both *in vivo* and *in vitro* studies, indicating the need for other similar studies.

ACKNOWLEDGMENTS

The authors are grateful to the Department of Ayurveda Yoga, Unani, Siddha and Homeopathy (AYUSH), Government of India, New Delhi, for granting Extra Mural Research project to ARKB; to the University of Kalyani for providing a Research Fellowship to SJB, to Dr. Taposh Das and Dr. Tapas Nag, Assistant Professors, All India Institute of Medical Sciences, New Delhi, for their help in electron microscopy, and to Dr. S.P. De, a former professor of Metropolitan Homeopathic Medical College, and Dr. D.N. De, Medical College, Kolkata, for encouragement.

REFERENCES

1. Daoust R, Molnar F. Cellular populations and mitotic activity in rat liver parenchyma during azo dye carcinogenesis. Cancer Res 1964;24:1898–1909.
2. Samuels AR, Bhargava M, Levine WG. Uptake of hepatobiliary fate of two hepatocarcinogens, N,N-dimethyl-4-aminoazobenzene and 3′-methyl-N,N-dimethyl-4-aminoazobenzene, in rat. Cancer Res 1983;43:4816–4821.
3. Kitagawa T, Sugano H. Enhancement of azodye hepatocarcinogenesis with dietary phenobarbital in rats. Gann 1977;68:255–256.
4. Biswas SJ, Khuda Bukhsh AR. Effect of homeopathiuc drug, Chelidonium, in amelioration of p-DAB induced hepatocarcinogenesis in mice. BMC Complement Altern Med 2002;2:4.
5. Biswas SJ, Khuda Bukhsh AR. Evaluation of protective potentials of a potentized homeopathic drug, Chelidonium majus, during azo dye induced hepatocarcinogenesis in mice. Indian J Exp Biol 2004;42:698–714.
6. Boericke W. Pocket Manual of Homeopathic Meteria Medica, Indian Edition. Calcutta, India: Sett Dey and Co., 1976.
7. Khuda Bukhsh AR. Towards understanding molecular mechanisms of action of homeopathic drugs: An overview. Mol Cell Biochem 2003;253:339–345.
8. Varma PN, Vaid I, eds. Encyclopaedia of Homoeopathic Pharmacopoeia. Vol I B. New Delhi, India: Jain Publishers, 2002:654–657.
9. Datta S, Mallick P, Khuda Bukhsh AR. Comparative efficacy of two microdoses of a potentized homeopathic drug, Cadmium Sulphoricum, in reducing cytogenetical effects produced by cadmium chloride in mice: A time-course study. BMC Complement Altern Med 2001;1:1–18.
10. Datta S, Mallick P, Khuda-Bukhsh AR. Efficacy of a potentized homeopathic drug (Arsenic Album-30) in reducing genotoxic effects produced by arsenic trioxide in mice: Comparative studies of pre-, post- and combined pre-and post-oral administration and comparative efficacy of two microdoses. Complement Ther Med 1999;7:62–75.
11. Datta S, Mallick P, Khuda-Bukhsh AR. Efficacy of a potentized homeopathic drug (Arsenic Album-30) in reducing genotoxic effects produced by arsenic trioxide in mice: II. Comparative efficacy of an antibiotic, actinomycin D alone and in combination with either of two microdoses. Comlement Ther Med 1999;7:156–163.
12. Biswas SJ, Pathak S, Khuda-Bukhsh AR. Assessment of genotoxic and cytotoxic potentials of an anti-epileptic drug, phenobarbital in mice. Mutat Res 2004;563:1–11.
13. Schmid W, Hollaender AE. eds. Chemical Mutagens: Principles and Methods of Detection. New York: Plenum Press, 1976:31–53.
14. Wyrobek AJ, Watchmaker G, Gordon L. Sperm morphology testing in mice. In: Kilbey BJ, Legator M, Nichols W, Ramel C, eds. Handbook of Mutagenicity Testing Protocols. New York: Elsevier Science, 1984:733–750.
15. Buege JA, Aust S. Microsomal lipid peroxidation. Methods Enzymol 1984;105:302–310.
16. Bergmeyer HU, Brent E. In: Bergmeyer HU, ed. Methods of Enzymatic Analysis, vol 2. New York: Academic Press, 1974:735–760.
17. Walter K, Schutt C. Acid and alkaline phosphatase in serum (two-point method). In: Bergmeyer HU, eds. Methods in Enzymatic Analysis, vol 2. New York: Academic Press, 1974: 856–860.
18. Kitagawa T, Pitot HC, Miller EC, Miller JA. Promotion by dietary phenobarbital of hepatocarcinogenesis by 2 methyl-N,N-

dimethyl-4-aminoazobenzene in the rat. Cancer Res 1979; 39:112–115.
19. Hathway DE. Mechanisms of chemical carcinogenesis. Oxford, UK: Butterworth, 1986:14–55.
20. Yamamoto R, Iishi H, Tatsuta M, et al. Inhibitory effect of sialoadenectomy on hepatocellular tumorigenesis in male mice induced by 3″-methyl-4- dimethylaminoazobenzene. Virchows Arch 1994; 425:79–82.
21. Caballero F, Gerez E, Oliveri L, et al. On the promoting action of tamoxifen in a model of hepatocarcinogenesis induced by p-dimethylaminoazobenzene in CF1 mice. Int J Biochem Cell Biol 2001;33:681–690.
22. Ohnishi S, Murata M, Degawa M, Kawanishi S. Oxidative DNA damage induced by an N-hydroxy metabolite of carcinogenic 4-dimethylaminoazobenzene. Jpn J Cancer Res 2001;92:23–29.
23. Vavreckova C, Gawlik I, Muller K. Benzophenanthridine alkaloids of *Chelidonium majus*: II. Potent inhibitory action against the growth of human keratinocytes. Planta Med 1996;62:491–494.
24. Vavreckova C, Gawlik I, Muller K. Benzophenanthridine alkaloids of *Chelidonium majus*; I. Inhibition of 5- and 12-lipoxygenase by a non redox mechanism. Planta Med 1996;62:397–401.
25. Khuda-Bukhsh AR. Potentized homeopathic drugs act through regulation of gene expression: A hypothesis to explain their mechanism and pathways of action *in vivo*. Complement Ther Med 1997;5:43–46.
26. Ernst E, Pittler MH, Stevenson C, White AR. The Desk Top Guide to Complementary and Alternative Medicine. Edinburgh, Mosby, 2001.
27. Ernst E. The current position of complementary/alternative medicine in cancer. Eur J Cancer 2003;39:2273–2277.
28. Weigant FAC, Van Rijn J, van Wijk R. Enhancement of the stress response by minute amounts of cadmium in sensitized Reuber H35 hepatoma cells. Toxicology 1997;116:27–37.
29. Fisher P. Research in Homeopathy—A Bibliography Compiled and Annotated by Dr. P Fisher, 8th ed. London: Royal London Homeopathic Hospital, 1992.
30. Linde K, Jonas WB, Worke DMF, et al. Critical review and meta-analysis of serial agitated dilutions in experimental toxicology. Hum Exp Toxicol 1994;13:481–492.
31. Linde K, Clausius N, Remirez G, et al. Overviews and meta-analyses of controlled clinical trials of homeopathy. In: Ernst E, Hahn EG, eds. Homeopathy: A Critical Appraisal. London: Butterworth-Heinemann, Reed Educational and Professional Publishing, Ltd., 1998.
32. Shen J, Anderson R, Albert PS. Use of complementary and alternative therapies by women with advanced stage breast cancer. BMC Complement Altern Med 2002;2:8–15.
33. Wilkinson S, Gomella LG, Smith JA. Attitudes and use of complementary medicine in men with prostrate cancer. J Urol 2002;168:2505–2509.
34. Chrystal K, Allan S, Forgeson G, Isaacs R. The use of complementary and alternative medicine by cancer patients in New Zealand and regional cancer treatment center. New Zealand Med J 2003;116:1–8.
35. Pathak Sen, Multani AS, Banerji P, Banerji P. Ruta 6 selectively induces cell death in brain cancer cells but proliferation in normal peripheral blood lymphocytes: A novel treatment for human brain cancer. Int J Oncol 2003;23:975–982.

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