Potentized homeopathic drug, Chelidonium-30 and Chelidonium-200, can ameliorate genotoxicity induced by p-Dimethylaminoazobenzene (p-DAB) in mice and modulate enzymatic activities in different organs during hepatocarcinogenesis


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Running Title: Homoeopathic drug, Chelidonium-30 and 200, shows antitumor and antimutagenic activities against induced liver cancer in mice

Keywords: DAB, hepatocarcinogenesis, microdoses chelidonium, genotoxicity

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ABSTRACT

Background

Crude extracts of various parts like root, shoot and leaves of *Chelidonium majus* (Papaveraceae) and also purified compounds derived from crude extracts of this plant have been reported to exhibit interesting anti-viral, anti-inflammatory, anti-tumor and anti-microbial properties both *in vitro* and *in vivo*. Extracts (mother tincture) and various homeopathic microdoses (potencies) of *Chelidonium majus* are routinely used by homeopathic practitioners against various liver disorders, including liver cancer in human with good effect. But, to our knowledge, no laboratory study has yet been conducted to examine whether homeopathic microdoses of Chelidonium can really exhibit any anti-tumor or anti-genotoxic activities in mice, a good mammalian model, after inducing liver cancer in them by administering hepatocarcinogenic agents. Therefore, in the present investigation, the efficacies of two microdoses (potencies) of the homeopathic drug Chelidonium, namely Chelidonium-30 (Ch-30) and Chelidonium-200 (Ch-200), in reducing tumor-formation in liver and in modulating genotoxicity and some enzymatic activities during p-Dimethylaminoazobenzene (DAB)-induced hepatocarcinogenesis in mice have been tested against suitable controls.

For this, several cytogenetical (e.g. chromosome aberration study, micronuclei testing, mitotic index, sperm head anomaly) and enzymatic protocols (e.g. acid and alkaline phosphatases, peroxidases) were used at three fixation intervals, viz., at 60 days, 90 days and 120 days of treatment. Different sets of healthy mice were fed: i) hepatocarcinogen, p-dimethylaminoazobenzene (p-DAB, initiator) plus phenobarbital (PB, promoter), ii) only PB, iii) neither p-DAB nor PB (normal control). One set of mice fed with DAB plus PB was also fed Ch-30 (iv) and another set Ch-200 (v).
Results

All group (i) mice developed tumors in liver at all fixation intervals, while none of group (ii) and (iii) mice developed any tumor. Some (about 40% in all) mice in group (iv) and group (v) did not show tumor nodules in their liver though all of them received the carcinogen in the same way as group (i) did. The frequencies of chromosome aberrations (CA), micronucleated erythrocytes (MN), mitotic index (MI) and sperm head abnormality (SHA) were much higher in group (i) than in both group (ii) and group (iii) mice (p<0.05 to 0.001) at all fixation intervals. However, in both group (iv) and (v) mice which received either potency of the homeopathic drug along with the carcinogen, the frequencies of CA, MN, SHA were much less than in (i) (p<0.05 to p<0.001) that did not receive the homeopathic drug.

Conclusion

Thus, both homeopathic potencies of Chelidonium exhibited anti-tumor and anti-genotoxic activities during azo dye-induced hepatocarcinoma in mice and also modulated favorably activities of marker enzymes like acid and alkaline phosphatases and peroxidases (p<0.05 to p<0.001) in them. The potentized Ch-30 and 200 having no visible ill effects of their own, may be strong candidates for use in delaying/protecting liver cancer.
BACKGROUND

*Chelidonium majus* L. (Papaveraceae) is a plant of great interest for its use in various diseases in European countries and in Chinese herbal medicines. Crude extracts of various parts like root, shoot and leaves have been reported to have several isoquinoline alkaloids, such as, sanguinarine, chelidonine, chelerythrine, berberine and coptisine 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 micro doses of Chelidonium herb extract are routinely used against several forms of liver disorders, including liver cancer [6] with good effect. But, to our knowledge, whether ultra-low doses of *Chelidonium majus*, namely, Chelidonium-30 (henceforth to be called Ch-30) and Chelidonium-200 (to be referred to as Ch-200), could also have similar anti-tumor or anti-genotoxic activities had not been experimentally tested so far in mice *in vivo*. The present investigation was therefore undertaken primarily to examine if Ch-30 and Ch-200, prepared as per homeopathic procedure, could show i) 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 marker enzymes like acid and alkaline phosphatases, and peroxidase in various tissues during azo dye induced hepatocarcinogenesis in mice.
MATERIALS AND METHODS

Materials

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

Preparation of the potentized homeopathic drug

The two potencies of Chelidonium, procured from “HAPCO”, 165, Bipin Behari Ganguli Street, Kolkata, were prepared as per the standard procedure of homoeopathic drug
preparation. The dry drug material of *Chelidonium majus* (whole plant) was extracted in 44% ethyl alcohol (i.e. the “mother tincture”). 1 ml of the mother tincture was subsequently diluted with 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**

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

**Feeding procedure and dose**

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

**Laboratory Methodology**

**Cytogenetic assay**

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

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

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

For sperm head anomaly (SHA), the epididymis of each side of mouse of both (control and treated) sets was dissected out and its inner content squeezed out into 10 ml of 0.87% normal saline separately. It was made free of fats, vas deferens and other tissues. The content was thoroughly shaken, filtered through a silken cloth and dropped on grease free clean slides. The slides were allowed to air dry and then stained by dilute Giemsa (1 ml Giemsa in 10 ml distilled water).

Biochemical assays

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

Estimation of Lipid Peroxidase

The lipid peroxidation was estimated from the supernatant by the method of Buege and Aust [8]. 1 ml of sample (homogenate containing 0.1-0.2mg of 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 535 nm in a double beam spectrophotometer against a suitable blank. The malonaldehyde concentration of the sample was calculated by using extinction coefficient of $1.56 \times 10^5 \text{ M}^{-1}\text{cm}^{-1}$.

**Estimation of Acid and Alkaline Phosphatases**

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

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

**Statistical analysis and scoring of data**

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

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

Cytogenetical Studies

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

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

Mitotic index

In both Ch-30 and 200 fed mice, the MI was much less than in the DAB plus PB fed mice and the protection was statistically significant (p<0.05 through p<0.001). The mitotic index in the PB fed mice was only slightly more than in the normal control series (table-1, Fig. 4).

Sperm head anomaly

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

Lipid peroxidase activity

The lipid peroxidase activity was the highest in all the three tissues at all fixation intervals in the DAB+ PB treated series except at 90 and 120 days in the liver where the activity was the highest in the DAB + PB+Ch-200 fed mice (Fig. 6-8). However, interestingly enough, while
the lipid peroxidase enzyme activity was generally much reduced in the Ch-200 fed mice as compared to Ch-30 fed mice in spleen and kidney, the lower micro dose i.e. Ch-30 appeared to reduce the activity in liver (p<0.05) more than that of Ch-200 at all fixation intervals (p<0.01 to p< 0.001, see table 2). The feeding of PB alone produced similar or marginally increased activity in spleen, kidney and liver at 60 and 90 days but the activity became appreciably higher in liver and kidney at 120 days but not in spleen.

**Alkaline phosphatase activity**

The alkaline phosphatase activity (AlkPA) in the DAB + PB fed mice was highest in spleen and liver at all the three fixation intervals while the activity declined in both Ch-30 and Ch-200 fed mice (p< 0.05, p<0.001, see table-2, Fig. 9-11). Ch-200 showed more ability in reducing AlkPA than that of Ch-30 fed mice except at 90 days in case of spleen. In the kidney, however, although DAB + PB showed, except for a few cases, a high degree of AlkPA at all fixation intervals, the activity could not be reduced by Ch-200 till at 120 day (p<0.05) while the activity level became actually higher at 60 and 90 days. However, Ch-30 could reduce the activity to some extent at all the fixation intervals although the differences were not statistically significant.

**Acid phosphatase activity**

The acid phosphatase activity (AcPA) in spleen, liver and kidney was very high in DAB + PB fed mice and the activity marginally declined in the Ch-30 and Ch-200 fed mice except at some fixation intervals where actually it was higher than in the DAB + PB fed mice. The differences were not statistically significant in most cases and only some favorable modulation was noted in liver and kidney at 120 days (p<0.05, p<0.001; see table 2, Fig. 12-14).
DISCUSSION

It had earlier been conclusively demonstrated that dietary PB had positive carcinogenic effect only when fed with the azodye 2-methyldiaminoazobenzene, but neither of these two when fed alone showed positive hepatocarcinogenesis in both mice and rat [10-12]. It is generally accepted that covalent binding of the metabolites of DAB (e.g. MAB, AAB etc.) with DNA is a major carcinogenic factor [13]. In the present study, the only PB fed mice also did not develop tumors in liver, while those fed with DAB + PB developed tumors. However, interestingly enough, 2 out of 5 mice, that is, about 40% of mice that received both DAB and PB along with either Ch-30 or Ch-200 did not develop tumors in liver while all mice fed DAB plus PB, but no Chelidonium developed tumors. This seems to be a dramatic finding as neither of these potentized drugs Ch-30 or Ch-200 had literally a single molecule of original drug substance in their diluted forms and were yet capable of reducing/delaying tumor growth in mice. Such agents that can antagonise or render protection at various levels of carcinogenesis are always considered very important, particularly so when they can be administered in micro doses and they do not have any ill-effects/side-effects of their own. When microdoses of either of these drugs were fed alone to healthy mice in similar doses, and dissected at corresponding fixation intervals, no tumor was found in their liver, nor was any genotoxic effect found from the assay of their chromosomes, spermheads, or micronuclei. A search of literature did not reveal any paper that dealt with such modulations of effects at the cytogenetic and biochemical levels during induced hepatocarcinogenesis in mice by the administration of the potentized Chelidonium although Roberfroid et al [14] reported that micro doses of PB 9C positively reduced the incidence of tumors and mortality in rats chronically fed with another carcinogen Acetylaminofluorene along with PB. However, these authors did not consider any of the protocols used in the present study. On the other hand, Fisher [15] reported that the DNA repair mechanisms of cultured mammalian
cells *in vitro* could be stimulated by very small doses of mutagens while working with human lymphocytes.

The observation of reduced frequencies of chromosome aberrations, MN and abnormal sperm head in the drug fed group of mice in the present study was also significant. There were supportive evidences of detoxification from the study of several enzyme activities 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 [16-19]. The favorable modulations of some of these enzymes, chromosomal and spermhead damages noted in the Chelidonium fed mice as compared to drug unfed DAB plus PB fed mice were some welcome features associated with the feeding of the micro doses of this drug. Therefore, from the results of the present investigation, it was suggestive that the micro doses of Ch i.e. 30 and 200 had positive protective effects against the hepatocarcinoma induced by DAB plus PB, and that Ch-200 appeared to have marginally better effects at the longer intervals. Incidentally, the efficacy of serial agitated dilutions of homeopathic drugs in experimental toxicology had also been convincingly advocated by meta-analysis done by Linde *et al* [20].

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

It is difficult to understand precisely at the present state of our knowledge how the ultra low doses of Chelidonium could achieve such spectacular protective changes which were amply demonstrable in the present study with the different protocols used. One hypothesis to explain the possible mechanism of action of the micro doses could be as follows. Since these low doses of the medicines were administered orally, their actions could possibly be mediated through the receptor systems located on tongue and the oral cavity. The drugs must have emanated specific signals in the receptor cells that could activate specific region of brain (presumably hypothalamus) in a manner that could possibly help elicit further signals to activate or repress certain transcriptional activities of specific regions of DNA meant for restoration of the damages caused due to the carcinogenic interaction. One way to test this hypothesis can be either to measure the activity of the signal transduction system through estimation of secondary messenger (i.e cyclic AMP) or else to block the pathway selectively after use of any drug in such microdoses. Incidentally, when some potentized homeopathic drugs were used along with Actinomycin D, a transcription blocker, the homeopathic drugs failed to elicit the desired protective levels against chromosomal and other cytogenetical damages produced by arsenic intoxication and by ultrasonic sound waves [21-22] as compared to when the drugs were administered alone (i.e. without Actinomycin-D). Thus the repair mechanisms were essentially mediated through active gene action. Khuda Bukhsh [23] proposed a hypothesis to explain the possible mechanism of action of such micro doses based on many circumstantial evidences [21-33] that the micro doses act through the regulation of relevant gene expression by eliciting impulses similar to that of some hormones and enzymes. That can suitably explain the mechanisms involved in the repair of damaged chromosomes or sperm head by the application of the micro doses. This can also
explain the antagonizing action against tumorigenesis/carcinogenesis which is essentially a multi-gene and multi-step process in majority of cancer including hepatocarcinoma [34]. Since the activation of proto-oncogene to oncogene is the key event for the transformation of the normal hepatocyte to a malignant liver tumor cell and that this process is controlled by the interactions of many tumorigenic and tumor supressor genes (e.g. p53 gene), it may be speculated that the micro doses of the drug might have interfered with the process of carcinogenesis either by actively modifying action of certain of these genes responsible for the transformation of cells to cancerous ones by release of specific transcription factors, or else by activating certain tumor-suppressor genes in an unknown manner. In fact the modulating effects of the drug on restoration of damage caused to several gene regulated phenomena like enzyme activities, chromosome and sperm structure etc would further strengthen the contention that these drugs possibly acted through regulatory actions on a number of key genes, related not only to the structure and normal functioning of liver hepatocytes, but also to the ones meant for maintaining integrity of bone marrow chromosomes and sperm head.

**CONCLUSION**

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

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REFERENCES


Explanation of Figures

**Figures 1a-m:** Photomicrographs of metaphase complements showing normal set of chromosomes (1a), and chromosome aberrations like break (B, 1b), acentric fragment (AF, 1c), terminal association (TA, 1d), ring (R, 1e), centric fusion and precocious centromeric separation (1f), C mitosis (1g) polyploidy (1h);

sperm with normal (1i), and abnormal (1j-1k) head morphology;

normo- (1l), and poly- (1m) chromatic erythrocytes.

Bar represents 10µm.

**Figure 2:** showing % of CA in different series of mice at different fixation intervals.

**Figure 3:** showing % of MNE in different series of mice at different fixation intervals.

**Figure 4:** showing % of MI in different series of mice at different fixation intervals.

**Figure 5:** showing % of SHA in different series of mice at different fixation intervals.

**Figure 6:** showing lipid peroxidase activity in liver of experimental mice at different fixation intervals.

**Figure 7:** showing lipid peroxidase activity in kidney of experimental mice at different fixation intervals.

**Figure 8:** showing lipid peroxidase activity in spleen of experimental mice at different fixation intervals.
**Figure 9:** showing alkaline phosphatase activity in liver of experimental mice at different fixation intervals.

**Figure 10:** showing alkaline phosphatase activity in kidney of experimental mice at different fixation intervals.

**Figure 11:** showing alkaline phosphatase activity in spleen of experimental mice at different fixation intervals.

**Figure 12:** showing acid phosphatase activity in liver of experimental mice at different fixation intervals.

**Figure 13:** showing acid phosphatase activity in kidney of experimental mice at different fixation intervals.

**Figure 14:** showing acid phosphatase activity in spleen of experimental mice at different fixation intervals.
Table: 1. Showing frequency distribution of mitotic indices (MI) in 5000 cells, chromosome aberration (CA) in 500 cells, micronuclei (MN) in polychromatic (PCE) and normochromatic (NCE) erythrocytes in 5000 cells and sperm head anomaly (SHA) in 5000 sperm in mice treated with p-Dimethyldiaminoazobenzene (DAB) + Phenobarbital (PB), DAB + PB + Chelidonium (Ch)-30 and DAB + PB + Chelidonium (Ch)-200 and their Phenobarbital treated and normal controls. Equal number of cells/sperms observed from each of 5 mice of a set.

<table>
<thead>
<tr>
<th>Fixation intervals (days)</th>
<th>Series</th>
<th>Mitotic Index</th>
<th>% of MI (% ± SE)</th>
<th>% of Prot.</th>
<th>Chromosome Aberration</th>
<th>% of Major CA</th>
<th>% of Other CA</th>
<th>Total CA (% ± SE)</th>
<th>% of Prot.</th>
<th>Micronucleated Erythrocytes</th>
<th>% of MN in PCE</th>
<th>% of MN in NCE</th>
<th>P/N</th>
<th>Total MN (% ± SE)</th>
<th>% of Prot.</th>
<th>Sperm Head Anomaly</th>
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<td>Mitotic Index</td>
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<td>Normal</td>
<td>1.32±0.08</td>
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<td>1.4</td>
<td>2.80±0.58</td>
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<td>0.26</td>
<td>0.89</td>
<td>0.16±0.05</td>
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<td>60 DAB + PB</td>
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<td>1.6</td>
<td>1.8</td>
<td>3.40±0.98</td>
<td>0.14</td>
<td>0.28</td>
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<td>DAB + PB + Ch-30</td>
<td>4.66±0.34</td>
<td>4.04b</td>
<td>4.4</td>
<td>4.6</td>
<td>9.0±0.78</td>
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<td>0.79</td>
<td>0.52±0.09</td>
<td>0.20</td>
<td>1.66±0.30</td>
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<td>1.82c</td>
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<td>DAB + PB + Ch-200</td>
<td>3.36±0.37</td>
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<td>12.8±3.87</td>
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<td>90 DAB + PB</td>
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<td>0.13</td>
<td>0.22</td>
<td>0.83</td>
<td>0.18</td>
<td>0.08</td>
<td>0.52±0.09</td>
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<td>DAB + PB + Ch-30</td>
<td>3.08±0.29</td>
<td>3.3a</td>
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<td>1.6</td>
<td>0.24</td>
<td>0.55</td>
<td>1.33</td>
<td>0.37±0.10</td>
<td>0.16</td>
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<td>3.5a</td>
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<td>0.48</td>
<td>1.16</td>
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<td>1.66±0.16</td>
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<td>1.4</td>
<td>1.4</td>
<td>2.80±0.58</td>
<td>0.26</td>
<td>0.26</td>
<td>0.89</td>
<td>0.16±0.05</td>
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<td>120 DAB + PB</td>
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<td>0.54</td>
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<td>0.58</td>
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<td>DAB + PB + Ch-200</td>
<td>8.72±0.65</td>
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<td>14.8±2.18</td>
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<td>0.27</td>
<td>0.34</td>
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<td>0.32</td>
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<td>14.4c</td>
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<td>2.18</td>
<td>0.14±0.06</td>
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Major Types of CA include: Break; Centric fusion; Translocation; Fragment; Pulverisation; Ring; Terminal association; Polyploidy; Aneuploidy.
Other types of CA include: Erosion, Constriction, Precocious centromeric separation, Centromeric stretching, Stickiness, C-mitotic effect.
SE= standard error, a= p<0.05, b= p<0.01, c= p<0.001.
Prot.= Protection given by the drug.
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<td>16.67</td>
<td>25.44 ± 1.43</td>
<td>15.85</td>
<td>0.09 ± 0.10</td>
<td>0.12</td>
<td>0.99 ± 0.02</td>
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<td>DAB + PB</td>
<td>263.20 ± 1.48</td>
<td>290.43</td>
<td>117.68 ± 1.89</td>
<td>115.30</td>
<td>0.11 ± 0.10</td>
<td>0.23</td>
<td>0.25 ± 0.02</td>
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<td>0.30 ± 0.05</td>
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<td>134.35 ± 1.23</td>
<td>133.45</td>
<td>117.68 ± 1.89</td>
<td>115.30</td>
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<td>0.23</td>
<td>0.25 ± 0.02</td>
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<td>172.35 ± 1.02</td>
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<td>0.25 ± 0.02</td>
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</table>

SE = Standard error, a = p<0.05, b = p<0.01, c = p<0.001.
Prot. = Protection given by the drug.
Figure 2
Figure 3
Figure 4
Figure 6
Figure 7
Figure 8
Figure 13