Original Article

Attenuation of p-dimethylaminoazobenzene initiated genotoxicity and cytotoxicity in mice by the combined treatment of a traditional homeopathic remedy Chelidonium Majus 200C and vitamin-C

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ABSTRACTS

The homeopathic remedy Chelidonium majus 200C (Chel-200) is traditionally used by homeopathic practitioners in liver ailments arising out of hepatotoxicity. The present investigation was aimed at examining whether vitamin C (L-ascorbic acid or AA), used in both traditional and orthodox medicines, can show better effects when used in combination with Chel-200, in favorably modifying the toxicological effects induced by the chronic feeding of p-dimethylaminoazobenzene (p-DAB, initiator) and phenobarbital (PB, promoter) in mice for 7 days through 120 days to induce hepatotoxicity and liver tumors. Mice were euthanized at 7, 15, 30, 60, 90, and 120 days of carcinogen feeding to assess various cytogenetical, biochemical and histological changes occurring in them. In a placebo controlled study, Chel-200 or the respective placebo (Alcohol-200C or Alc, "vehicle" of homeopathic drug), was orally administered to toxicant-fed mice. Sub-groups of the mice receiving Chel-200 were also fed either AA or an Alc placebo and their individual and conjoint effects were studied against the respective controls, to evaluate if the combination therapy of Chel-200 with AA can be of additional help in the amelioration of the toxicities generated by the toxicants. The combined feeding of Chel- 200 and AA appeared to reduce the cytotoxic and genotoxic effects significantly, when compared to either only the Chel-200 or AA fed group. A similar trend was also obtained in the results of scanning and transmission electron microscopic studies of the livers. Experiments in other mammalian models are warranted to confirm if these drugs in combination could be used in palliative care of human patients with liver diseases including cancer.

Keywords p-dimethylaminoazobenzene, hepatocarcinogenesis, ultra-high dilution, L-ascorbic acid, homeopathy, Chelidonium Majus 200C

INTRODUCTION

Homeopathy is a popular form of alternative medicine which has traditionally been used as a cost-effective, non-toxic and simple mode of treatment by a large number of people around the world, particularly in India and the neighboring countries, for more than two hundred fifty years now. One of the basic tenets of homeopathy is "like cures like", meaning thereby that the symptoms generated by a healthy person through chronic consumption of a substance in higher doses, can also be ameliorated by the micro doses of that substance in ultra-high dilution. In homeopathy, two forms of medicines are used: i) the mother tinctures, mostly comprising of the ethanolic extract of some medicinal plant- or animal-parts, or minerals, and ii) the potentized or dynamized forms that comprise of serially diluted and agitated forms of the mother tinctures with ethyl alcohol (70 - 90%); the latter forms, when homeopathically diluted to 10^{-23} and above following the centesimal scale, cross the Avogadro's limit and the remedies do not contain a single molecule of the original drug substance, raising serious questions as to how it can act as a remedy without carrying any molecular presence of the drug substance in it. Therefore, it becomes necessary to examine if such ultra-high diluted remedies can realistically alter or modulate certain demonstrable and quantifiable traits and if they do, whether the changes or modulations are in favor of the recovery process. Since cancer is a dreadful disease where there is still need for a composite approach of various medical systems, the present study was designed in such a way as to integrate the benefits of ultra-high remedies of homeopathy. Chelidonium Majus 200C (Chel-200), which is successfully used to alleviate the suffering arising out of hepato-toxicity and some other related issues, to a routinely used anti-oxidant therapy with vitamin-C, which can be obtained readily from some dietary components also, in getting better responses in the amelioration of induced hepatoto-toxicity and hepatic tumors. In integrative cancer therapy, drugs of traditional systems which have minimal toxic effects or side-effects of their own, which have proven or validated abilities of assisting in the recovery process, are favored for use as supportive medicine to provide a better way of life to patients, particularly those suffering from liver cancer with high rate of mortality.

Chelidonium majus (commonly known as greater celandine) is a herbaceous plant reaching from 30 to 120 cm in height. It belongs to the family Papavaraceae. It is used as a strong liver medicine by practitioners of traditional and folk medicines. The

main alkaloid present in the herb and root is coptisine. Other alkaloids present include methyl 2'-(7, 8-dihydrosanguinarine-8-yl) acetate, allocryptopine (Cahlikova et al., 2010) stylopine, protopine, norchelidonine, berberine, chelidonine, sanguinarine, chelerythrine (Li et al., 2011) and 8-hydroxydihydrosanguinarine (Park et al., 2011). Of these, sanguinarine is particularly toxic.

Various epidemiological and experimental studies suggested that diets rich in micronutrients generally reduce the risk of cancer and mutation (Ames, 1983; Wenzel et al., 2004). DNA damage from micronutrient deficiencies has been suggested as one of the major causes of cancer and therefore studies involving vitamin supplementation are numerous. Various epidemiological, experimental, and metabolic studies have shown that nutrition can play an important preventive role in the initiation, promotion, and progression stages of several types of cancer (Levine, 1986; Sauberlich, 1984). Many cancer chemopreventive agents obtained from natural resources possess antioxidant potential and are non-toxic (Kaegi, 1998; Kaegi, 1998; Kaya, 2003; Wattenberg, 19920). Various studies have been conducted in recent years to investigate the genotoxic and anti-genotoxic properties of L-ascorbic acid (AA), both *in vitro* and *in vivo*, though on a lesser scale on the *in vivo* system. AA L-ascorbic acid is known to play an important role in the synthesis of collagen, to promote wound healing and to influence many immunological and biochemical reactions in the body (Kaya, 2003). It is also considered to be one of the most potent and least toxic antioxidants for humans and studies have suggested that ascorbic acid may protect the integrity of cell membranes, promote cell differentiation and the inhibition of tumour growth and increased survival of animals with implanted tumours (Alcain and Buron, 1994; Frei and Ames, 1989; Meadows et al., 1991; Prasad, 1980). Complementary and alternative medicine practitioners worldwide currently use ascorbate because there is no apparent harm (Biswas and Khuda-Bukhsh, 2007; Jackson et al., 1995; Moore et al., 1985). Ascorbic acid (AA) is important micronutrient which functions as a factor in several metabolic reactions. Free radicals cause DNA strand breaks and chromosome deletions and rearrangements and activated oxygen species play an important role in tumor promotion and progression. The azo dye used in the present investigation is used as a coloring agent for polishes and soap and evaluated as a Group-2B carcinogen by the International Agency for Research on Cancer (IARC) which acts as an initiator of hepatocarcinogenesis; on the other hand, PB is a widely used sedative and an antiepileptic drug which acts as a promoter (Biswas and Khuda-Bukhsh, 2004, 2005; Biswas and Khuda-Bukhsh, 2005). Further, crude extracts of *Chelidonium majus* and purified compounds derived from it, have been reported to have antiviral, antitumor and antimicrobial properties, also it has been previously reported by us that crude plant extracts and various homeopathically potentized forms of Chelidonium Majus have hepato-protective abilities (Biswas et al., 2008; Biswas and Khuda-Bukhsh, 2001; Colombo and Bosisio, 1996). However, the crude extract being toxic in nature means it has to be cautiously used and the quantity is advised to be used in micro doses (Teschke et al., 2011, 2012). Previously we have also reported that AA can modulate the toxicity induced by p-DAB+PB (Biswas and Khuda-Bukhsh 2004). Various studies have already been carried out separately with AA, and Chelidonium as an anticancer drug; however, no systematic study seems to have been carried out with any mammalian model to examine whether AA in combination with Chel-200 can really act favorably when administered against p-DAB-induced toxicity in mice. Therefore, using this initiation–promotion model, the present study was undertaken

and the effects of p-DAB+PB on several tissues of mice were studied; simultaneously the modulating effect of AA along with ultrahigh diluted Chel-200 was also undertaken and here we report the protective ability of AA in combination with Chel-30 in mice by using several cytogenetical and histological protocols as well as by testing some biomarkers of wide scientific acceptability.

MATERIALS AND METHODS

Experimental design
The experimental protocol was approved by the Institutional Animal Ethical Committee (IAEC), University of Kalyani and the care of the laboratory was taken as per the CPCSEA regulation. Swiss albino mice *(Mus musculus)*, weighing about 25 ± 2 g, were reared and maintained in the animal house of the Department at a temperature of $26 \pm 3°C$ with 12/12 h light/dark cycle throughout the experiment. Mice were divided into 6 sets comprising of 45 mice each. A group of mice (n = 45) were fed food and water *ad libitum* that served as normal controls. This was Group I. Another set of mice (n = 45) were fed 0.06% p-dimethylaminoazobenzene (p-DAB, D-6760, SIGMA) at a daily dose of 165 mg/kg b.w. per mouse mixed with food along with 0.05% phenobarbital (PB) 0.03 mg per mouse everyday. This was Group II. The third group (Group III) of mice (n = 45) was fed the same as group II along with 0.06 ml of 1% L-ascorbic acid [SRL, India] and Chelidonium 200 [Chel-200]. The fourth set (Group IV) of mice (n = 45) was also fed similarly as in Group II along with Chel-200. The fifth set (Group V) of mice were fed as in Group II along with 0.06 ml of 1% L-ascorbic acid. The sixth set (Group VI) of mice were fed with diluted alcohol and served as the positive control.

Preparation of the ultrahigh diluted drug
The potency of Chelidonium-200 was procured from HAPCO, 165 BB Ganguly street, Kolkata, prepared as per the standard procedure, the dry drug material of Chelidonium majus (whole plant) was extracted with 44% ethyl alcohol (i.e. mother tincture). The mother tincture (1 ml) was subsequently diluted with 99 ml HPI approved ethanol solvent (IP 96/Homeopathic Pharmacopoeia of India approved grade ethyl alcohol) and succussed 10 times with mechanical strokes of equal force to make 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/shakes and the procedure was repeated to get the ultra-high dilution of Chel-200. Thus, in theoretical terms, there could not be any measurable scale to represent the presence of any material drug in the remedy, being diluted 10^{60} times and even a single molecule of the original drug substance of Chelidonium extract could not be expected to be present, but only a few of its nanoparticles or "molecular imprints" in the aquatic ethanol could be present. Since the potency 200 was made from an ethanolic crude extract of Chelidonium majus (not from initial dried powder of the extract), it was not possible to quantify the absolute/final concentration of the diluted drug; therefore, in homeopathy, the dilution is expressed conventionally in terms of its dilution rather than quantifying the final concentration in terms of its nanoscale or picogram scale.

Preparation of stock solution of the drug and dilute alcohol
Chel-200 was finally diluted separately with 20 ml of double distilled water to make the stock solution of the drug; 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 0.06 ml of either Chel-200 alcohol from the stock solutions at a time with the aid of a fine pipette as per the requirement of the particular series.

Laboratory Methodology
Mice were injected ip with 0.03% colchicine solution at the rate of 1 ml/100 gm bw about 1 h and 15 min before being killed. The limb bones were quickly dissected out from the anesthetized mice and the bone marrow cells collected and dispersed in 1% sodium citrate solution kept at 37℃. After about 10 min, the bone marrow cells were spun down at 1000 × g for 6 - 8 min and fixed in freshly prepared aceto-alcohol (acetic acid 1: ethyl alcohol 3). Slides for the chromosomal assay were prepared by the conventional flame drying technique followed by Giemsa staining (Biswas et al., 2004, Biswas et al., 2008). A total of 500 bone marrow cells were observed, either 100 from each of 5 mice or 50 from each of 10 mice (longer intervals) of a set.

For micronucleus (MN) preparation, clean grease free slides with smeared bone marrow cells were 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.

The mitotic index (MI) was assessed from the same slide that was scanned for MN. The non-dividing and dividing cells were recorded and their ratios calculated following Schmid (1976), i.e. Mitotic Index = Number of dividing cells /Number of non-dividing cells.

For sperm head anomaly (SHA) study, the technique of Wyrobek (19834) was adopted with minor modification, the epididymis of each side was dissected out and the inner contents was taken out into 10 ml of 0.9% normal saline, it was made free of fats, vas deferens and other tissues. The contents were thoroughly shaken and filtered through a silken cloth and dropped on clean grease free slides. The slides were allowed to air dry and then stained by Giemsa (1 ml Giemsa in 10 ml of distilled water).

Biochemical assays
Mice were sacrificed and their liver, were quickly isolated and separately homogenized with cold 0.87% normal saline, followed by centrifugation at 3000 g for 20 min at 4℃ in a cooling centrifuge (C24-BL, Remi Instruments, India). Before carrying out the enzymatic estimations, the quantitative estimation of total protein was made by the method of Lowry et al. (1951).

The lipid peroxidation was estimated from the supernatant by the method of Buege and Aust (1984). Briefly 1 ml of sample (homogenate containing 0.1-0.2 mg of protein) was mixed thoroughly with 2 ml of TCA-TBA-HCl (15% w/v TCA and 0.375% w/v TBA in 0.25 N HCl). The absorbance of the sample was determined at 535 nm in a double beam spectrophotometer against a suitable blank. The malonaldehyde concentration of the sample was calculated by using an extinction coefficient of 1.56×10^5 M^{-1}/cm^{-1}.

For estimation of AST and ALT, the methods of Bergmeyer and Brent (1974) were followed. Briefly for aspartate aminotranferase (AST), 0.1 ml 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℃.This was followed by the addition of 0.5 ml of dintrophenylhydrazine (DNPH) and then by 5.0 ml 0.4 N NaOH. The absorbance was measured at 510 nm.

For the analysis of alanine amino transferase (ALT), 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℃. The rest of the procedure was same as that of AST and the absorbance was measured at 510 nm.

For the study of acid phosphatase (ACP) and alkaline phosphatase (ALP), the method of Walter and Schutt (1974) was followed. Briefly for ACP, 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, p-nitrophenyl phosphate. For ALP activity 0.05 ml of tissue homogenate was mixed with 2 ml alkaline buffer so that the volume always stood at 2.05 ml. It was incubated at 37℃ for 30 min, then 10 ml of 0.05 N NaOH was added and the absorbance was measured at 405 nm against a blank.

For preparation of histological slides of liver at day 60 and 120, the standard methodology using Bouin's fixative and microtome sectioning has been followed.

For scanning electron microscopy (SEM) of liver at day 60 and 120, the standard gold coating technique using critical point-drier (CPD-Biorad, Microscience Division, Warford England), sputter-coater (Agar Sputter Coater, Model 198, Stansted, United Kingdom) etc was adopted in case of scanning electron microscopy (LEO, 435VP, United Kingdom).

For transmission electron microscopy, (TEM CM-10, Philips Microscope) 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 of each of five different mice at each fixation interval were analyzed.

Statistical analysis and scoring of data
The significance test between different series of the data was conducted by student t-tests. Homogeneity of the different series was further analyzed by the oneway ANOVA (Analysis of Variance) using SPSS 11.0 version. Groups indicated by a, b or c are significantly different. Though tests were conducted for
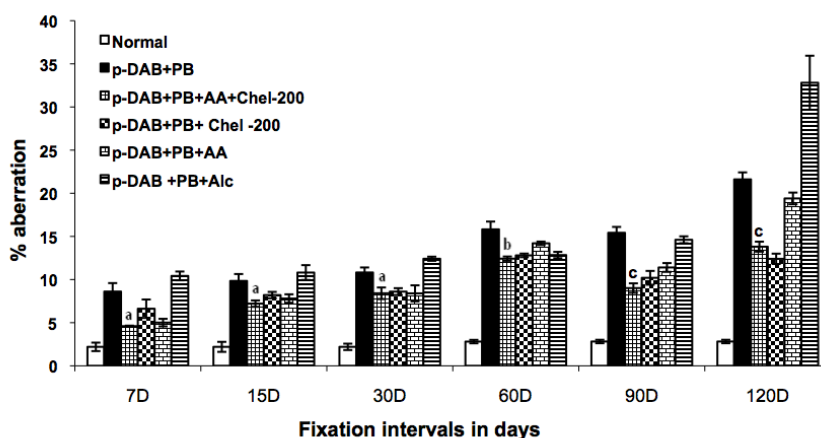
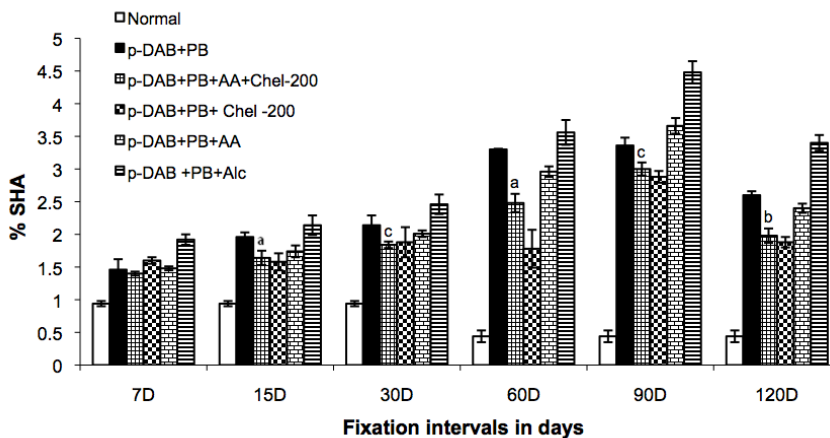**Fig. 1. Percentage of chromosome aberration** Showing the major and minor aberrations (CA) in different series of control and treated mice at different fixation intervals. Major CA includes breaks, translocation, pulverization, ring, terminal association, and centric fusion. Minor aberration includes precocious centromeric separation, polyploidy, streatching. a: $p < 0.05$, b: $p < 0.01$, c: $p < 0.001$.
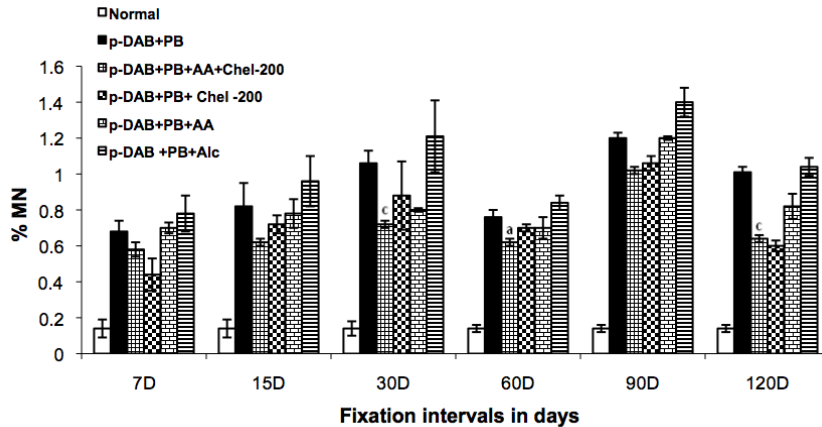
Fig. 2. Percentage of MN in different series Showing the distribution of micronucleated erythrocytes (MN) in different series of control and treated mice at different fixation intervals. a: $p < 0.05$, b: $p < 0.01$, c: $p < 0.001$.
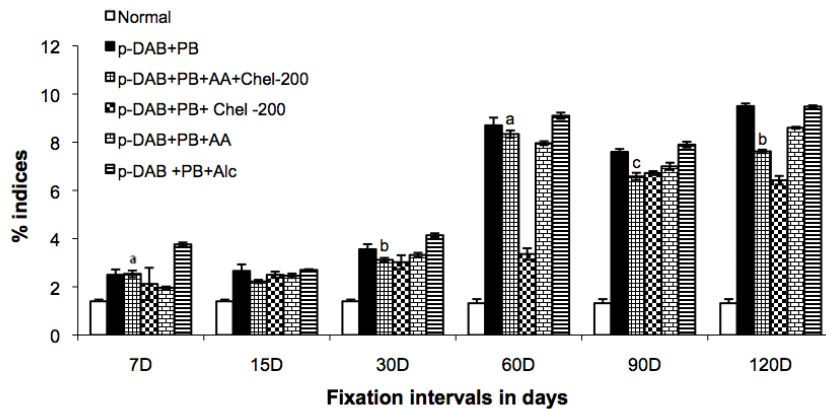
Fig. 3. Percent of mitotic indices in different series Showing the mitotic indices (MI) in different series of control and treated mice at different fixation intervals. a: $p < 0.05$, b: $p < 0.01$, c: $p < 0.001$.

Fig. 4. Percentage of SHA at different fixation inbtervals Showing the sperm head abnormality (SHA) in different series of control and treated mice at different fixation intervals. a: $p < 0.05$, b: $p < 0.01$, c: $p < 0.001$.

significance values for normal vs. p-DAB+PB or normal vs. p-DAB+PB+Alc, these were not included in the table as this would only make it more cumbersome. Since we are more concerned with the differences between p-DAB+PB+Alc versus p-DAB+PB+AA+Chel-200 only, we represented the significant differences between them in the table.

RESULTS

Chromosome aberrations studies
As compared with normal metaphase complements there was a gradual increase in CA in the p-DAB+PB- and p-DAB+PB+ Alc-fed mice at all fixation intervals except at day 90. However, when the data of CA of the p-DAB+PB+AA+Chel-200 fed mice was compared with that of p-DAB+PB+ Alc, it revealed that there was a significant decrease of CA at all fixation intervals and they were statistically significant ($p < 0.05$ to $p < 0.001$, Fig.1). Further, the data of DAB+PB+AA+Chel-200 was compared with that of DAB+PB+AA and DAB+PB+Chel-200. There was a significant decrease in CA at most fixation intervals.

Micronucleated erythrocytes
Data on the occurrence of MN in polychromatic and normochromatic erythrocytes have been provided in Fig. 2. The percentages of MN were high in the p-DAB+PB+ Alc-fed mice at all fixation intervals when compared to normal and p-DAB+PB, p-DAB+PB +AA, p-DAB+PB+Chel-200-fed series; however in the p-DAB+PB+AA+Chel -200-fed mice it was appreciably reduced (statistically significant) at 15, 30, 60, 90 days intervals when compared to p-DAB+P B+AA, and p-DAB+PB+ Chel-200 ($p < 0.05$ through $p < 0.001$).

Mitotic Index
The mitotic indices were significantly elevated in the p-DAB+PB+ Alc fed mice at all fixation intervals when compared to normal and the p-DAB+PB, p-DAB+PB+AA and p-DAB+PB+Chel-200 fed series. When compared to p-DAB+PB+AA, in p-DAB+PB+Chel-200 there was a significant decrease in mitotic indices in the p-DAB+PB+AA+Chel-200 fed mice at 15, and 90 days. ($p < 0.05$ to $p < 0.001$, Fig. 3).

Sperm head anomaly
Quite a high incidence of sperm head abnormality was recorded in the p-DAB+PB+ Alc-fed mice at all fixation intervals when compared to the normal and p-DAB+PB fed series. The percentages of sperm with abnormal head morphology was considerably reduced in the p-DAB+PB+AA+Chel-200-fed series at all fixation intervals when compared to p-DAB+PB+ Alc-fed series ($p < 0.05$ to $p < 0.001$, Fig. 4), one important observation was that SHA was reduced in p-DAB+PB+AA+Chel-200-fed series, but this decrease was less than in the p-DAB+PB+Chel-200-fed series.

Table 1. Mean activities of various enzymes in different tissues of control and treated mice at fixation intervals

Fixation intervals (days)	Series	LPO			AST			ALT			ACP			ALP		
		L	K	S	L	K	S	L	K	S	L	K	S	L	K	S
7	Normal	9.12 ± 0.51	6.15 ± 0.81	10.1 ± 1.0	0.08 ± 0.01	0.16 ± 0.02	0.06 ± 0.01	0.034 ± 0.01	0.032 ± 0.012	0.031 ± 0.05	0.04 ± 0.01	0.08 ± 0.004	0.10 ± 0.001	0.06 ± 0.01	0.07 ± 0.004	0.091 ± 0.001
	p-DAB+PB	35.25 ± 1.77	39.51 ± 0.88	36.20 ± 1.06	0.53 ± 0.006	0.41 ± 0.059	0.44 ± 0.012	0.504 ± 0.01	0.12 ± 0.01	0.162 ±0.003	0.736 ±0.016	0.757 ± 0.003	0.863 ± 0.001	0.588 ± 0.009	1.748 ± 0.021	0.139 ± 0.007
	p-DAB+PB+AA+Chel-200	33.99 ± 2.97[b]	45.84 ± 0.41[b]	23.48 ± 0.535[a]	0.34 ± 0.002	1.03 ± 0.08[c]	0.05 ± 0.017[a]	0.114 ± 0.002[a]	0.11 ± 0.015[b]	0.051 ± 0.005	0.55 ± 0.003[a]	0.226 ± 0.001[c]	0.416 ± 0.002[a]	0.052 ± 0.001[b]	0.576 ± 0.02	0.071 ± 0.008[b]
	p-DAB+PB+ Chel -200	54.23 ± 2.25	31.84 ± 2.42	28.68 ± 4.45	0.45 ± 0.002	0.70 ± 0.004	0.26 ± 0.008	0.144 ± 0.001	0.05 ± 0.001	0.11 ± 0.001	0.64 ± 0.008	0.29 ± 0.005	0.54 ± 0.001	0.018 ± 0.001	0.403 ± 0.001	0.018 ± 0.002
	p-DAB+PB+AA	34.25 ± 1.22	34.47 ± 0.75	23.33 ± 0.25	0.41 ± 0.007	0.36 ± 0.006	0.15 ± 0.005	0.287 ± 0.003	0.068 ± 0.003	0.064 ± 0.001	0.52 ± 0.001	0.39 ± 0.01	0.76 ± 0.003	0.54 ± 0.004	1.11 ± 0.023	0.077 ± 0.005
	p-DAB +PB+Alc	43.05 ± 0.93	47.06 ± 0.55	41.63 ± 1.02	0.53 ± 0.009	0.38 ± 0.01	0.30 ± 0.004	0.630 ± 0.001	0.13 ± 0.002	0.152 ± 0.004	0.74 ± 0.012	0.77 ± 0.008	0.85 ± 0.003	0.58 ± 0.009	1.71 ± 0.12	0.138 ± 0.004
15	Normal	9.11 ± 0.51	6.15 ± 0.81	10.1 ± 1.0	0.08 ± 0.01	0.16 ± 0.02	0.06 ± 0.01	0.034 ± 0.01	0.032 ± 0.012	0.031 ± 0.05	0.04 ± 0.01	0.08 ± 0.004	0.10 ± 0.001	0.06 ± 0.01	0.07 ± 0.004	0.091 ± 0.001
	p-DAB+PB	57.66 ± 0.56	34.52 ± 4.17	33.85 ± 1.84	0.69 ± 0.006	0.97 ± 0.005	0.44 ± 0.013	0.481 ± 0.002	0.19 ± 0.004	0.095 ± 0.004	0.73 ± 0.016	0.78 ± 0.005	0.778 ± 0.007	0.57 ± 0.005	1.73 ± 0.04	0.152 ± 0.004
	p-DAB+PB+AA+Chel-200	28.05 ± 3.04[c]	17.45 ± 4.85[a]	32.67 ± 4.44[a]	0.41 ± 0.06[a]	0.99 ± 0.008[a]	0.42 ± 0.005	0.36 ± 0.002	0.167 ± 0.005[a]	0.082 ± 0.003	0.448 ± 0.011[b]	0.482 ± 0.006[a]	0.48 ± 0.025[a]	0.438 ± 0.011[a]	1.028 ± 0.023[c]	0.144 ± 0.003
	p-DAB+PB+ Chel -200	21.41 ± 8.05	10.15 ± 1.11	39.17 ± 7.49	0.36 ± 0.008	0.84 ± 0.016	0.39 ± 0.008	0.38 ± 0.007	0.16 ± 0.005	0.08 ± 0.003	0.47 ± 0.004	0.55 ± 0.002	0.66 ± 0.01	0.511 ± 0.011	1.49 ± 0.02	0.13 ± 0.008
	p-DAB+PB+AA	15.47 ± 0.17	26.91 ± 8.46	27.04 ± 0.84	0.66 ± 0.001	0.69 ± 0.003	0.35 ± 0.007	0.49 ± 0.011	0.115 ± 0.007	0.071 ± 0.001	0.42 ± 0.001	0.55 ± 0.004	0.57 ± 0.01	0.53 ± 0.007	0.98 ± 0.001	0.132 ± 0.001
	p-DAB +PB+Alc	69.96 ± 0.18	31.53 ± 0.66	42.92 ± 6.41	0.78 ± 0.007	1.24 ± 0.008	0.44 ± 0.008	0.54 ± 0.003	0.24 ± 0.003	0.09 ± 0.001	0.78 ± 0.002	0.79 ± 0.001	0.82 ± 0.002	0.61 ± 0.005	1.82 ± 0.013	0.15 ± 0.007
30	Normal	9.11 ± 0.51	6.15 ± 0.81	10.1 ± 1.0	0.08 ± 0.01	0.16 ± 0.02	0.06 ± 0.01	0.034 ± 0.01	0.032 ± 0.012	0.031 ± 0.05	0.04 ± 0.01	0.08 ± 0.004	0.10 ± 0.001	0.06 ± 0.01	0.07 ± 0.004	0.091 ± 0.001
	p-DAB+PB	87.89 ± 2.92	54.41 ± 1.01	68.15 ± 0.89	1.20 ± 0.006	3.21 ± 0.02	0.41 ± 0.001	0.81 ± 0.01	0.42 ± 0.007	0.14 ± 0.003	0.85 ± 0.001	0.72 ± 0.002	0.78 ± 0.004	0.94 ± 0.06	1.12 ± 0.034	1.13 ± 0.014
	p-DAB+PB+AA+Chel-200	43.02 ± 0.85[b]	35.27 ± 1.74[c]	62.25 ± 0.74[a]	0.51 ± 0.007[b]	0.64 ± 0.006[a]	0.28 ± 0.005	0.368 ± 0.013[b]	0.089 ± 0.002[c]	0.060 ± 0.002[a]	0.60 ± 0.038[a]	0.44 ± 0.002[b]	0.64 ± 0.005[a]	0.165 ± 0.002[b]	0.558 ± 0.001[a]	0.908 ± 0.019[c]
	p-DAB+PB+ Chel -200	39.99 ± 0.54	45.99 ± 1.177	91.79 ± 0.761	0.68 ± 0.013	1.25 ± 0.01	0.17 ± 0.002	0.704 ± 0.006	0.124 ± 0.003	0.052 ± 0.002	0.67 ± 0.032	0.387 ± 0.002	0.502 ± 0.004	0.226 ± 0.004	0.383 ± 0.024	1.30 ± 0.008
	p-DAB+PB+AA	82.9 ± 1.22	45.46 ± 0.566	62.31 ± 0.878	0.65 ± 0.005	0.86 ± 0.01	0.27 ± 0.007	0.49 ± 0.01	0.105 ± 0.002	0.08 ± 0.002	0.67 ± 0.011	0.24 ± 0.01	0.483 ± 0.012	0.29 ± 0.008	0.304 ± 0.003	0.641 ± 0.033
	p-DAB +PB+Alc	69.02 ± 1.64	60.61 ± 1.38	90.01 ± 2.93	1.28 ± 0.003	1.07 ± 0.007	0.35 ± 0.003	0.83 ± 0.012	0.165 ± 0.002	0.147 ± 0.013	0.739 ± 0.013	0.979 ± 0.007	0.925 ± 0.005	0.936 ± 0.068	1.115 ± 0.034	1.129 ± 0.014

L- Liver, K-Kidney, S-Spleen. The different levels of statistical significances between p-DAB +PB+Alc and p-DAB+PB+AA+CH-200 have been designated by a: $p < 0.05$, b: $p < 0.01$, c: $p < 0.001$. lipid peroxidation-LPO, aspartate aminotransferase (AST), alanine aminotransferase (ALT) in mM/min/mg, acid phosphatase (ACP), alkaline phosphatase (ALP) in mM phenol liberated/100 mg protein.

Table 1. (Cont.) Mean activities of various enzymes in different tissues of control and treated mice fixation intervals

Fixation intervals (days)	Series	LPO			AST			ALT			ACP			ALP		
		L	K	S	L	K	S	L	K	S	L	K	S	L	K	S
60	Normal	9.11 ± 0.51	6.15 ± 0.81	10.1 ± 1.0	0.08 ± 0.01	0.16 ± 0.02	0.06 ± 0.01	0.034 ± 0.01	0.032 ± 0.012	0.031 ± 0.05	0.04 ± 0.01	0.08 ± 0.004	0.10 ± 0.001	0.06 ± 0.01	0.07 ± 0.004	0.091 ± 0.001
	p-DAB+PB	101.1 ± 11.5	70.30 ± 2.19	74.51 ± 1.33	0.539 ± 0.01	1.18 ± 0.01	0.19 ± 0.004	0.55 ± 0.03	0.19 ± 0.006	0.06 ± 0.002	0.81 ± 0.008	1.28 ± 0.017	1.51 ± 0.012	0.049 ± 0.019	0.54 ± 0.01	1.14 ± 0.03
	p-DAB+PB+AA+Chel-200	59.12 ± 1.13[b]	49.22 ± 0.34[a]	51.99 ± 1.65	0.52 ± 0.008[a]	0.85 ± 0.06[b]	0.20 ± 0.003	0.36 ± 0.012[a]	0.107 ± 0.008	0.057 ± 0.004	0.702 ± 0.028[a]	0.835 ± 0.015[b]	0.517 ± 0.004[c]	0.191 ± 0.012[a]	0.280 ± 0.001[b]	1.124 ± 0.071
	p-DAB+PB+ Chel -200	67.48 ± 1.26	50.1 ± 1.64	67.74 ± 2.71	1.09 ± 0.01	0.51 ± 0.007	0.21 ± 0.004	0.69 ± 0.013	0.083 ± 0.005	0.061 ± 0.002	1.087 ± 0.101	0.561 ± 0.007	0.604 ± 0.006	0.259 ± 0.014	0.578 ± 0.04	1.059 ± 0.019
	p-DAB+PB+AA	66.20 ± 1.31	57.12 ± 0.70	63.07 ± 2.68	0.83 ± 0.008	0.56 ± 0.008	0.23 ± 0.01	0.57 ± 0.005	0.08 ± 0.002	0.068 ± 0.005	1.026 ± 0.044	0.608 ± 0.017	0.663 ± 0.011	0.37 ± 0.029	0.189 ± 0.006	1.123 ± 0.026
	p-DAB +PB+Alc	82.30 ± 1.85	78.20 ± 0.69	90.10 ± 2.11	0.78 ± 0.028	1.066 ± 0.01	0.22 ± 0.003	0.55 ± 0.01	0.19 ± 0.005	0.061 ± 0.003	1.025 ± 0.009	1.092 ± 0.037	1.442 ± 0.015	0.708 ± 0.034	0.477 ± 0.006	1.291 ± 0.011
90	Normal	9.11 ± 0.51	6.15 ± 0.81	10.1 ± 1.0	0.08 ± 0.01	0.16 ± 0.02	0.06 ± 0.01	0.034 ± 0.01	0.032 ± 0.012	0.031 ± 0.05	0.04 ± 0.01	0.08 ± 0.004	0.10 ± 0.001	0.06 ± 0.01	0.07 ± 0.004	0.091 ± 0.001
	p-DAB+PB	67.38 ± 5.1	45.17 ± 1.74	71.02 ± 0.73	0.77 ± 0.011	0.877 ± 0.02	0.41 ± 0.029	0.61 ± 0.006	0.188 ± 0.008	0.119 ± 0.003	0.728 ± 0.018	0.637 ± 0.007	0.602 ± 0.01	0.71 ± 0.014	0.669 ± 0.022	0.367 ± 0.011
	p-DAB+PB+AA+Chel-200	41.43 ± 1.74[b]	34.20 ± 1.11[a]	38.47 ± 0.06[b]	0.86 ± 0.01	0.87 ± 0.007	0.36 ± 0.011[a]	0.69 ± 0.01	0.15 ± 0.008	0.09 ± 0.005[c]	0.74 ± 0.014[b]	0.93 ± 0.024	0.92 ± 0.035[c]	0.17 ± 0.01[b]	0.39 ± 0.027[a]	0.39 ± 0.008
	p-DAB+PB+ Chel -200	43.69 ± 4.32	33.94 ± 4.18	34.51 ± 1.11	0.97 ± 0.012	0.63 ± 0.01	0.34 ± 0.015	0.79 ± 0.003	0.134 ± 0.007	0.1 ± 0.003	0.59 ± 0.022	0.57 ± 0.018	0.56 ± 0.03	0.39 ± 0.02	0.98 ± 0.037	0.302 ± 0.002
	p-DAB+PB+AA	56.76 ± 0.92	33.58 ± 1.03	78.81 ± 1.28	0.95 ± 0.005	0.91 ± 0.009	0.38 ± 0.009	0.76 ± 0.008	0.15 ± 0.018	0.113 ± 0.007	0.26 ± 0.015	0.48 ± 0.013	0.266 ± 0.02	0.26 ± 0.028	0.35 ± 0.017	0.278 ± 0.004
	p-DAB +PB+Alc	94.66 ± 5.68	51.89 ± 3.25	78.20 ± 0.42	0.67 ± 0.019	0.99 ± 0.026	0.54 ± 0.06	0.49 ± 0.006	0.22 ± 0.016	0.172 ± 0.01	1.44 ± 0.13	0.79 ± 0.04	1.58 ± 0.01	0.74 ± 0.016	0.92 ± 0.001	0.41 ± 0.007
120	Normal	9.11 ± 0.51	6.15 ± 0.81	10.1 ± 1.0	0.08 ± 0.01	0.16 ± 0.02	0.06 ± 0.01	0.034 ± 0.01	0.032 ± 0.012	0.031 ± 0.05	0.04 ± 0.01	0.08 ± 0.004	0.10 ± 0.001	0.06 ± 0.01	0.07 ± 0.004	0.091 ± 0.001
	p-DAB+PB	97.53 ± 0.41	92.41 ± 0.7	63.79 ± 0.52	0.87 ± 0.001	0.78 ± 0.001	0.33 ± 0.002	0.69 ± 0.006	0.18 ± 0.004	0.11 ± 0.003	1.103 ± 0.001	0.81 ± 0.002	0.75 ± 0.004	0.76 ± 0.001	0.83 ± 0.001	0.29 ± 0.003
	p-DAB+PB+AA+Chel-200	48.05 ± 0.449[b]	31.72 ± 0.26[a]	26.46 ± 0.99[a]	0.58 ± 0.004[b]	0.85 ± 0.002	0.3 ± 0.001[a]	0.49 ± 0.001[a]	0.15 ± 0.004[b]	0.088 ± 0.002[a]	0.71 ± 0.002	2.02 ± 0.01[c]	0.268 ± 0.001[c]	0.501 ± 0.002[b]	0.409 ± 0.001[b]	0.281 ± 0.001
	p-DAB+PB+ Chel -200	56.15 ± 0.344	34.92 ± 1.25	46.51 ± 0.17	0.79 ± 0.002	1.21 ± 0.004	0.23 ± 0.001	0.63 ± 0.005	0.27 ± 0.005	0.075 ± 0.001	0.75 ± 0.006	0.84 ± 0.001	0.48 ± 0.001	0.31 ± 0.002	0.61 ± 0.008	0.31 ± 0.001
	p-DAB+PB+AA	54.92 ± 0.18	48.10 ± 0.23	47.33 ± 0.38	1.10 ± 0.002	0.71 ± 0.002	0.46 ± 0.002	0.18 ± 0.002	0.118 ± 0.002	0.14 ± 0.002	1.06 ± 0.005	0.92 ± 0.002	0.64 ± 0.004	1.16 ± 0.004	0.398 ± 0.003	0.32 ± 0.002
	p-DAB +PB+Alc	69.28 ± 0.75	68.92 ± 1.43	43.58 ± 0.79	0.76 ± 0.003	0.68 ± 0.003	0.43 ± 0.004	0.61 ± 0.002	0.16 ± 0.002	0.155 ± 0.004	0.69 ± 0.002	0.63 ± 0.005	0.74 ± 0.005	0.902 ± 0.002	0.73 ± 0.002	0.255 ± 0.001

L- Liver, K-Kidney, S-Spleen. The different levels of statistical significances between p-DAB +PB+Alc and p-DAB+PB+AA+CH-200 have been designated by a: $p < 0.05$, b: $p < 0.01$, c: $p < 0.001$. lipid peroxidation-LPO, aspartate aminotransferase (AST), alanine aminotransferase (ALT) in mM/min/mg, acid phosphatase (ACP), alkaline phosphatase (ALP) in mM phenol liberated/100 mg protein.

Biochemical assays

Lipid peroxidation steadily increased from day 7 to day 60 and decreased at day 90, and again increased at day 120 in the p-DAB+PB-fed series when compared to the normal control. A similar trend was also noticed in the p-DAB+PB+Alc-fed series at all fixation intervals. However, in the DAB+PB+AA+Chel-200-fed mice, the activity always kept low at all fixation intervals when compared to the p-DAB+PB and p-DAB+PB+Alc fed series. The lipid peroxidation in the liver of p-DAB+PB+AA+Chel-200 remained significantly lower ($p < 0.05$ through $p < 0.001$, Table 1) than the p-DAB+PB+Chel-200, p-DAB+PB+AA alone fed series at 60, 90 and 120 days fixation intervals. However, the activity was lower significantly ($p < 0.05$ through $p < 0.001$) at 30, 120 and 60, 120 in the kidneys and spleen respectively (Table 1).

The activity of AST in the liver remained higher in the p-DAB+PB+Alc-fed series when compared to p-DAB+PB and normal controls at all fixation intervals. However, it is interesting to note that the AST activity in the livers of p-DAB+PB+AA+Chel-200 fed mice remained significantly lower ($p < 0.05$ through $p < 0.001$) than that of either p-DAB+PB+Chel-200- or p-DAB+PB+AA-fed series at all fixation intervals except after the 15th day (Table 1). In kidney and spleen the activity was significantly lower ($p < 0.05$ through $p < 0.001$) at 30, 90, 120 and 60, 90 and 120 days fixation intervals (Table 1).

The activity of ALT in the livers remained high in the p-DAB+PB+Alc-fed series when compared to that of the p-DAB+PB-fed and normal controls at all fixation intervals except at day 90. However, it is interesting to note that the activity in p-DAB+PB+AA+Chel-200 remained lower than that of the p-DAB+PB+Alc and p-DAB+PB fed series at all fixation intervals except at day 90 where the activity was higher. The activity of ALT in the liver in the p-DAB+PB+AA+Chel-200 was significantly lower ($p < 0.05$ through $p < 0.001$, Table 1) at 15, 30, 60, 90 days when compared to either p-DAB+PB+AA- or p-DAB+PB+Chel-200-fed series ($p < 0.05$ through $p < 0.001$) (Table 1).

The ACP activity in the liver was lower in the p-DAB+PB+AA+Chel-200-fed series when compared to p-DAB+PB+Alc and p-DAB+PB, at all fixation intervals except at day 90. However, it is interesting to note that the activity in p-DAB+PB rose higher than p-DAB+PB+Alc, fed series at day 90. The ACP activity in the normal control series was always lower at all fixation intervals when compared to other series. The activity of ACP in the liver in the p-DAB+PB+AA+Chel-200 was significantly lower ($p < 0.05$ through $p < 0.001$, Table 1) at 30, 60, 90 and 120 days when compared to the p-DAB+PB+AA and p-DAB+PB+ Chel-200 series. While in the kidneys the activity of ACP in p-DAB+PB+AA+Chel-200 fed series it was lower in 7 and 15 days only while in spleen the activity of p-DAB+PB+AA+Chel-200 was significantly lower at 15, 60 and 120 days (Table 1).

The ALP activity in liver was higher in the p-DAB+PB+Alc-, p-DAB+PB-fed series at all fixation intervals when compared to the normal control and p-DAB+PB+AA+Chel-200-fed series. The activity of ALP in the liver in the p-DAB+PB+AA+CH-200 was significantly lower ($p < 0.05$ through $p < 0.001$)) at 15, 30, 60, 90 and 120 days when compared to the p-DAB+PB+AA, and p-DAB+PB+ Chel-200 series (Table 1), but in the kidneys and spleen, the ALP activity in p-DAB+PB+AA+Chel-200 was lower than p-DAB+PB+ Chel-200 but not in the p-DAB+PB+AA series in longer fixation intervals (Table 1).

Histological studies

Analysis of the SEM study of the livers revealed that fibrosis was evident in the hepatic cells and there was massive necrosis of the tissue causing the appearance of holes and the blood liver
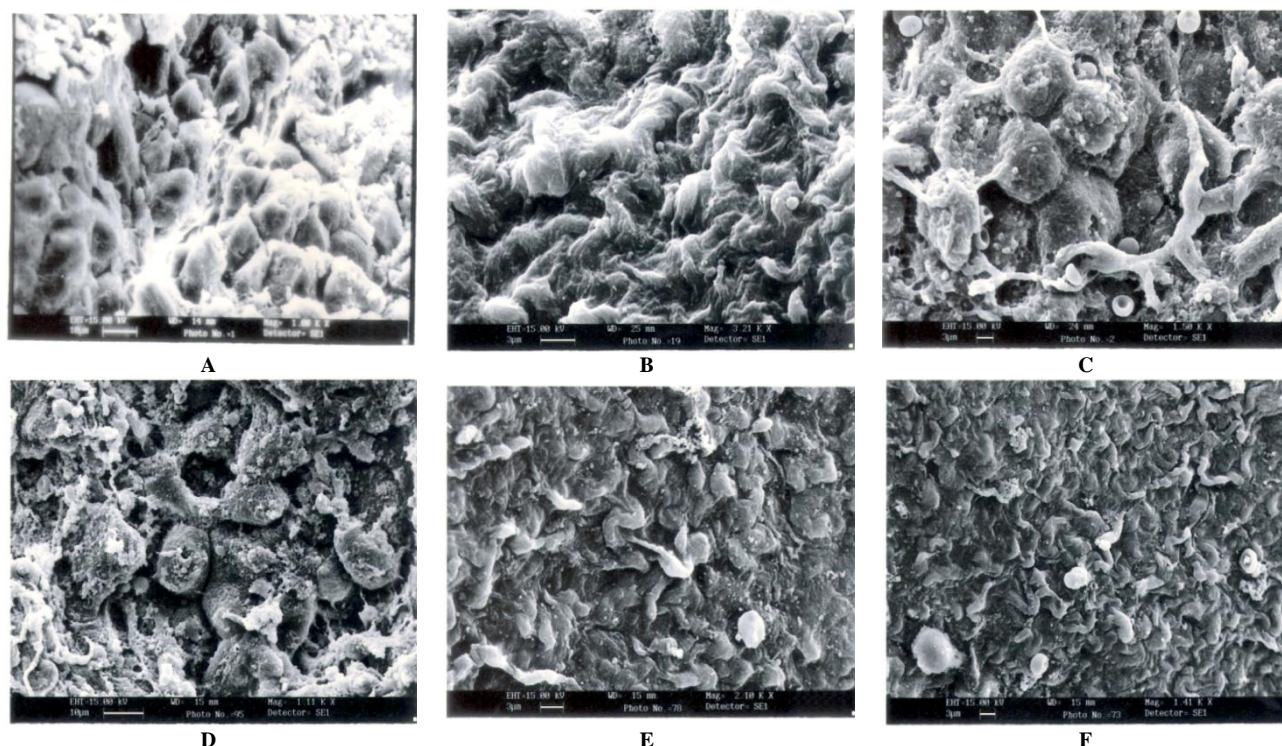
Fig. 5. (A). SEM of liver showing polyhedral cells with definite arrangements in normal control series; (B) SEM of liver sections showing fibrosis and massive necrosis in p-DAB+PB+Alc fed series; (C) showing loss of hepatc structure and edamatous swelling of hepatic cells in p-DAB+PB fed series; (D) showing SEM samples of hepatic tissue where the normalcy is restored with hepatic structures but lesser amount of necrosis in p-DAB+PB+AA+CH fed series; (E) showing hepatic structures where the amount of destruction was less but there is swelling of hepatic cells and lesser amount of tissue necrosis in p-DAB+PB+AA fed series; (F) SEM sections of p-DAB+PB+CH fed series.
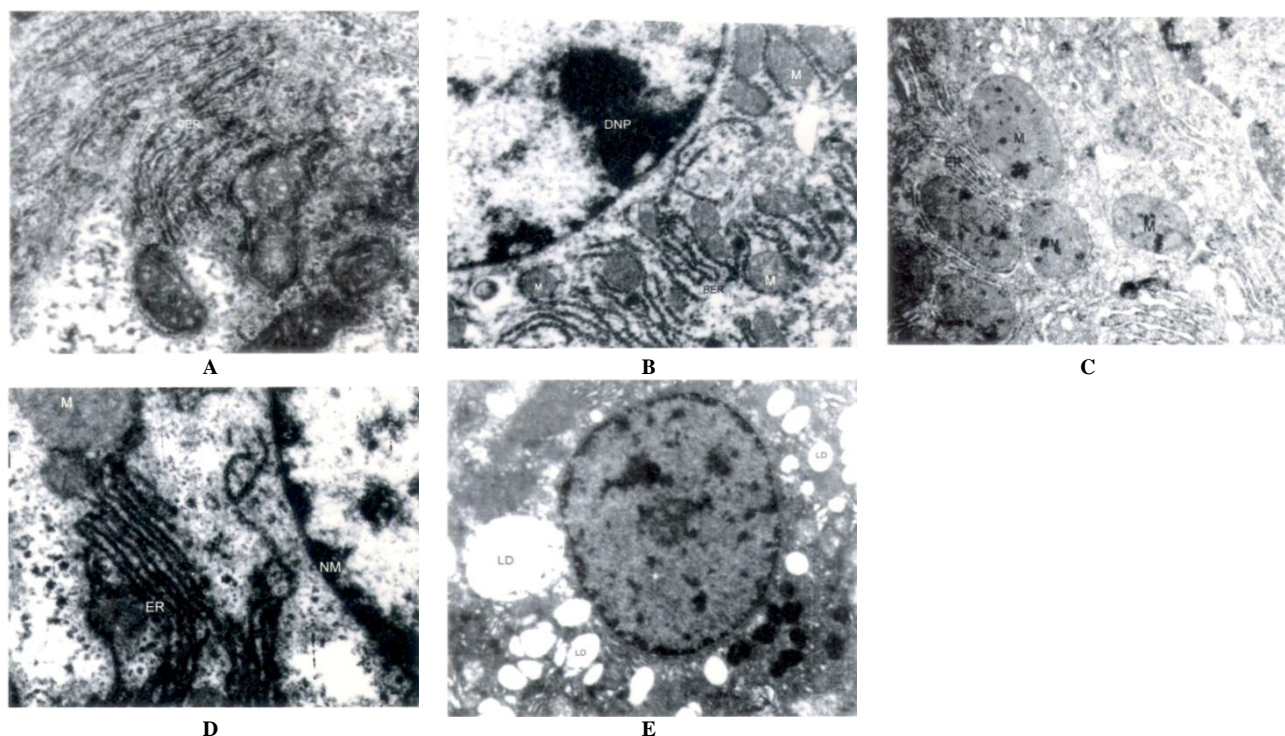
Fig. 6. (A) Representative photographs of normal liver section seen under Transmission Electron microscope where the cells contains continuous ER (endoplasmic reticulum), uniform mitochondria with their original structures; (B), (C) in the p-DAB+PB+Alc and p-DAB+PB fed series, ER were broken and discontinuous, numerous mitochondria (M) without any distinct shape, Kupffer cells were activated, lipid droplets were more in number and dispersed nucleoplasm (DNP); (D) in p-DAB+PB+AA+CH fed series normal was restored with continuous ER, nuclear membrane, mitochondria lesser in number when compared to p-DAB+PB+Alc and p-DAB+PB fed series; (E) TEM photographs of hepatic cells where the lipid droplets and mitochondria were present in p-DAB+PB+CH and p-DAB+PB+AA fed series but the extent of damage was less when comared to positive control.

barrier was not present in the p-DAB+PB+Alc- and p-DAB+PB-fed mice. Further, RBCs were also found in the parenchyma which would suggest the breakdown of the blood liver barrier. In the p-DAB+PB+AA+Chel-200-fed series, fibrosis was present but at a lower scale when compared to p-DAB+PB+Alc- and p-DAB+PB-fed mice, the edematous swelling was also less evident when compared to the p-DAB+PB+AA- or p-DAB+PB+Chel-200-fed series (Fig. 5A-F).

Hepatic examination by transmission electron microscopy revealed damage to intracellular organelles in the p-DAB+PB+Alc- and p-DAB+PB-fed series, a few notable changes were: the ER were broken and discontinuous, numerous mitochondria without any distinct shape were present, Kupffer cells were activated, lipid droplets were more in number, the activity of Kupffer cells might suggest an increased secretion of lymphokines. In the p-DAB+PB+AA+Chel-200-fed series a few notable changes were: the ER became continuous, lipid droplets, mitochondrias and Kupffer cells were lesser in number when compared to p-DAB+PB+Alc- or p-DAB+PB-fed series and these changes were more pronounced/clear when we compared them with that of p-DAB+PB+AA- or p-DAB+PB+Chel-200-fed series (Fig. 6A-E).

DISCUSSION

From the findings of our present study, it was evident that when Chel-200 was administered to carcinogen intoxicated mice, the manifestation of the hepatotoxic effect was invariably ameliorated in respect of all the parameters of study. This would clearly demonstrate the ability of the ultra-high diluted potentized homeopathic remedy in altering or modulating the parameters of study well accepted in the scientific community as standard protocols of a study. It was further revealed that the addition of the vitamin C to the homeopathic remedy actually attenuated the toxicity to a higher level of protection. Thus, the combined therapy apparently had a cumulative effect in terms of bringing benefit to the carcinogen-fed mice.

On the other hand, the simultaneous feeding of p-DAB and PB produced toxicity in all mice treated for 60 days or more. It is generally accepted that p-DAB is metabolically degraded to MAB (n-methyl-4-aminobenzene) and then to 4-aminobenzene which can bind to various liver proteins and also to DNA. This binding is believed to be a major clastogenic factor and carcinogenic factor (Ohnishi, 2001). The oral administration of p-DAB+PB and p-DAB+PB+Alc brought forth not only chromosomal damage to a considerable extent but also resulted in an elevated frequency of micronuclei (mainly derived from broken part or whole chromosome) and caused an increase in the mitotic index of the bone-marrow cells. The occurrence of chromosome aberrations, specially the breaks, would indicate that p-DAB might have acted after the duplication of chromosomes at the G_2 phase of the cell cycle. The sub-chromatid breaks that were encountered from day 7 to day 120 would also indicate that p-DAB had a delayed and lingering genotoxic effect. The other aberrations in the present investigation may be due to the existence of some vulnerable or relatively weaker spots on the chromosomes, which could actually represent the hotspots of p-DAB activity as suggested by Murata et al. (1999).

Amino azodyes have an exocyclic amino group that undergoes biochemical N-oxidation and further conversion to reactive electrophiles which produces deleterious reactive oxygen species or lipid peroxides. The production of free radicals were alleviated by the combined feeding of AA and Chel-200, which was reflected by a significant decline in LPO

content, ACP, ALP, AST and ALT activities in mice at most of the fixation intervals. The process of lipid peroxidation is initiated by the attack of a free radical on unsaturated lipids and the resulting chain reaction is terminated by the production of lipid breakdown products, lipid, alcohols, aldehydes and malonaldehyde. Thus, there is a cascade of peroxidative reactions leading to hepatotoxicity, which ultimately results in the destruction of lipid; hence lipid peroxidation was steadily increased from day 7 to 120 in both p-DAB+PB- and p-DAB+PB+Alc-fed mice. Ascorbic acid is an antioxidant that acts as a scavenger for a wide range of reactive oxygen species (ROS). Both tumour metastasis and cell migration have been correlated with the intracellular ROS level, so it was postulated that the inhibitory effect of ascorbic acid on cell motility may be caused by the scavenging of ROS (Block, 1991; Halliwell, 2001). Thus, in the present investigation the combined therapy has a better ameliorating potential than those which are treated only with Chel-200 and AA and the differences were significant in most of the treatment intervals. The results corroborated well with other experimental studies where ascorbic acid inhibits tumor growth, metastasis and acts as an antioxidant (Lee, 2002).

The micronucleus (MN) assay in is one of the most widely used methods for measuring DNA damage in toxicology which originates mainly from chromosome breaks or whole chromosomes that fail to engage with the mitotic spindle when the cell divides. In the present investigation, the inhibition of micronucleus formation was considerably evident in the p-DAB+PB+AA+Chel-200-fed series in most fixation intervals when compared to the only AA- and Chel-200- fed series. The spermatotoxic effects of p-DAB in germ cells of mice were evident from the increasing frequency of sperm with abnormal head morphology. This indicates that the cytotoxic effect of the azo-dye was also manifested in the germ cells. Since the major constitutents of Chelidonium extracts, chelidonine, sanguinarine and chelerythrine, are potent inducers of apoptosis. Ascorbic acid inhibits the malignant infiltrative growth, and possibly for the inhibition of tumor enzymes that promote invasiveness and are also believed to essential for the normal integrity and function of testis i.e. synthesis, development and maintenance of normal sperm (Dawson, 1990). Thus, in the present study there was a considerable reduction in abnormal sperm heads in the p-DAB+PB+AA+Chel-200-fed series when compared to the p-DAB+PB+AA- and p-DAB+PB+Chel-200 -fed series.

Incidentally, from the findings of the present study, a fairly strong correlation can be substantiated between modulations in the cytogenetical endpoints and the enzyme biomarkers, which were consistent in their expected patterns of expression, both in the p-DAB+PB-treated series as well as in the drug-fed series, but the modulations were more pronounced in p-DAB+PB+AA+Chel-200-fed series than those treated singularly, thereby depicting an overall positive influence of the remedy in ameliorating the toxic effects of p-DAB+PB in mice. Thus, the results of these investigations would also suggest that combined therapies (ultra-diluted remedies and AA) could also be tested for their possible positive role in the amelioration of p-DAB+PB toxicity in other mammals as well, and to confirm if the combined therapy could give more a protective ability than the single treatment.

How the ultra low doses of the remedy, which are not theoretically expected to possess even a single molecule of the original drug substance, could bring about multiple changes in both cytogenetical and enzyme biomarkers, is rather unclear at the present state of our knowledge. Incidentally, Khuda-Bukhsh (Khuda-Bukhsh, 2003, 2006, 2008) proposed a hypothesis that one mechanism through which the potentized homeopathic

drugs act could be through regulation of the expressions of certain relevant genes, by acting as suitable molecular switches with the ability to trigger a cascade of gene actions, as all the cytogenetical and biochemical markers tested are under the strict control of a specific genetic regulatory mechanism that is responsible for causing epigenetic modifications. Further, the ability of potentized homeopathic medicines to modulate expressions of signal proteins and receptor proteins (like AhR, PCNA) may provide additional evidence for their ability to influence the regulatory genes (Bhattacharyya et al., 2010). However more accurate and specific studies should be carried out to map exactly the points of cellular changes and similar experiments in other mammalian models are warranted to suggest if ultra-diluted drugs in combination with L-ascorbic acid could be used in palliative care in human cases of liver ailments including cancer.

CONFLICTS OF INTERSET

No authors/co-authors have any competing interest to declare.

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