The Egyptian Journal of Medical Human Genetics (2015) **16**, 301–312

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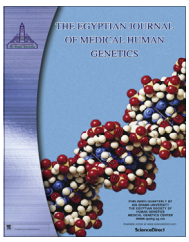

ORIGINAL ARTICLE

Differential response of biochemical parameters to EMS and MMS treatments and their dose effect relationship on chromosomes in induced diabetic mouse

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Received 13 April 2015; accepted 14 May 2015
Available online 10 June 2015

KEYWORDS

EMS;
MMS;
Chromosomal aberrations;
Diabetic mouse;
Catalase;
Glycogen;
MDA

Abstract *Aim:* To study the effect of alkylating agents such as EMS and MMS on chromosomes and biochemical parameters in induced diabetic mouse.

Methods: Chromosome preparations from bone marrow was made using the method of Evans et al. (1964) and biochemical estimations from the liver was done by the method of Sinha (1972) for catalase, Van der Vies (1954) for glycogen and Uchiyama and Mihara (1978) for MDA.

Results: The study has revealed that EMS and MMS induced a dose dependent increase in chromosomal aberrations of chromatid type in the diabetic mouse. Nonetheless, it is interesting to note that, there is significant reduction in the frequency of chromosomal aberrations in diabetic compared to non diabetic mice at all tested doses of EMS or MMS and at different recovery times [RTs]. On the other hand biochemical parameters showed a variable degree of reactivity: (1) catalase activity was significantly elevated in non diabetics whereas in diabetics it is significantly decreased with increasing concentrations of EMS. Contrary to this, the catalase activity in the case of MMS treatment is significantly reduced in non diabetics compared to diabetic mice. (2) However glycogen level is significantly reduced in both the diabetic and non diabetic with increasing concentrations of EMS or MMS, but MDA levels were significantly increased.

Conclusion: (1) Even though alkylating agents induce chromosomal aberrations in diabetic mice, MMS, a methylating agent is a more potent inducer of chromatid type of aberrations than EMS, an ethylating agent. (2) Diabetic mouse is more resistant than the non diabetic to alkylating agents and (3) the tested agents altered the analyzed biochemical parameters.

Abbreviations: EMS, ethyl methanesulfonate; MMS, methyl methanesulfonate; MDA, malondialdehyde; Stz, streptozotocin; TBA, thiobarbituric acid.

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Peer review under responsibility of Ain Shams University.

http://dx.doi.org/10.1016/j.ejmhg.2015.05.004

1. Introduction

Diabetes is one among the top ten leading killers of human population which is characterized by the deficiency in insulin production or inability of cells to detect insulin, resulting in increased blood glucose [1]. It was reported that about 382 million people are suffering from diabetes around the world and expected to reach 592 million by 2035 (World Diabetic Congress, 2013). About 26.9% of all people over 65 years have diabetes and 60% have cancer. Overall, 8–18% of cancer patients have diabetes [1]. Epidemiological studies of Lee et al. [2,3] have revealed that the incidence of diabetes has increased due to exposure to pollutants. Alkylating agents are one such group of pollutants which are affecting the genetic systems of animals including humans [4] on the one hand, and on the other, these are being used as anticancer agents [5]. Even though, alkylating agents are being extensively used for cancer treatment, their influence in diabetic conditions in humans in particular or other animal systems in general has not been worked out. *In vivo* human as test system cannot be used; hence mouse as model for diabetic condition has been employed to understand the influence of alkylating agents. It is worthy to mention that, there are extensive reports on the induction of chromosomal aberrations by alkylating agents in various *in vivo* test systems like Yeast [6], Plant [7,8], *Drosophila* [9], *Poecilocerus pictus* [10], non diabetic mouse [11,12], non diabetic rats [13] and *in vitro* test systems like Chinese hamster ovary cells [14,15] and Human lymphocytes [16,17]. Mahadimane and Vasudev [18,19] working in *in vivo* Ehrlich ascites, a disease condition, have demonstrated a dose dependent increase in clastogenicity using MMS or EMS. Though voluminous reports on the effects of alkylating agents in normal conditions are available, there is no literature as far as we know on diabetic animal systems. Hence, the present studies were undertaken to understand the influence of mono functional alkylating agents, such as EMS and MMS on chromosomes and biochemical parameters in induced diabetic mice.

2. Materials and methods

2.1. Chemicals

Alkylating agents, EMS (CAS number 62-50-0) and MMS (CAS Number 66-27-3) were purchased from Sigma Co. St. Louis, MO, USA and colchicine (CAS No. 64-86-8) from Himedia Pvt Ltd., Mumbai, India. Giemsa stain and other chemicals were of analytical grade commercially available. Streptozotocin (STZ) (CAS No. 18883-66-4) was purchased from SRL, Mumbai, India.

2.2. Animals

Male Swiss albino mice weighing 25–30 g of 6–8 weeks old were used. The mice were maintained in the laboratory, Department of Studies in Zoology, University of Mysore, Mysore, housed in polypropylene cages which were provided with standard feed pellets and water *ad libitum* under 12 h light/dark cycle. The study was approved by the Institutional animal ethics committee and the work was carried out in accordance with The Code of Ethics of The World Medical Association for experiments in animals.

2.3. Induction of diabetes in mouse

Diabetes was induced by injecting a single intra peritoneal dose of STZ (180 mg/kg body weight) (Freshly prepared in 0.1 M citrate buffer pH 4.5) as described by Yanardag et al. [20]. The control mice were given 0.5 ml of citrate buffer. Prior to administration, mice were fasted for 4 h but were given water *ad libitum*. Animals were kept under observation for 5 days following administration and blood glucose concentration was measured by SD check glucometer, Japan. On the fifth day mice with blood glucose levels above 300 mg/dl and below 400 mg/dl were used in the present studies as type I diabetic mouse [21].

2.4. Treatment schedule

Diabetic and non diabetic mice were grouped into six (A–F) in MMS and eight (A–H) groups in the case of EMS and each group consists of 3 mice. Diabetic and non diabetic mice were injected with 0.5 ml saline containing different concentrations of EMS (50, 100, 200, 250, 300, 350 and 400 mg/kg body weight) or MMS (35, 70, 105, 140, 175 mg/kg body weight). The controls received only 0.5 ml of saline. Animals were killed at 24, 48 and 72 h recovery times (RTs) and further processed for different parameters.

2.5. Mitotic chromosome preparation

90 min before killing, the treated animals were injected intraperitonealy with 0.5 ml of 0.05% Colchicine. Bone marrow was extracted and slides were prepared by a routine air dry technique [22]. These air- dried slides were coded and stained with 4% Giemsa for 20–30 min. Non-overlapping metaphase plates were scored for chromosomal aberrations such as chromatid breaks, chromatid exchanges, intra-chromatid deletions, triradials, chromosome breaks, dicentrics, rings, and minutes. 100 well spread metaphase plates were scored for each animal and experiments were repeated thrice.

2.6. Biochemical assays

After killing the animals at different RTs, the liver was dissected for biochemical assays such as catalase, glycogen and Malondialdehyde (MDA).

2.6.1. Activity of catalase (EC 1.11.1.6)

The activity of catalase was measured in liver homogenate by the method of Sinha [23]. The enzyme extract was added to the reaction mixture containing 1 ml of 0.01 M phosphate buffer (pH 7.0) and 0.5 ml of 0.2 M H_2O_2 for 30 s. The reaction was terminated by the addition of dichromate acetic acid. The formed chromic acetate was measured at 590 nm.

2.6.2. Estimation of glycogen

Total glycogen content was estimated according to the method of Van der Vies [24]. In brief, liver homogenate was prepared

Table 1 Percentage frequency of chromosomal aberrations (Mean ± SE) induced by different doses of EMS in non diabetic and diabetic mice recovered after 24 h.

Dose (mg/kg body wt)		B′	B″	RB′	RB′B″	ID	Ring	Minutes	Chromosomal aberration	Total number of breaks
Control I	Non diabetic	1.67 ± 0.19						0.56 ± 0.11	2.22 ± 0.22	2.22 ± 0.22[a]
Control II	Diabetic	1.22 ± 0.22	0.11 ± 0.11					1.11 ± 0.22	2.45 ± 0.40	2.56 ± 0.44[a]
50	Non diabetic	1.33 ± 0.38	0.33 ± 0.00					1.22 ± 0.11	2.89 ± 0.29	3.22 ± 0.29[a]
	Diabetic	1.11 ± 0.22	0.44 ± 0.29					1.56 ± 0.11	3.11 ± 0.11	3.56 ± 0.29[a]
100	Non diabetic	11.22 ± 0.40	1.56 ± 0.29	0.67 ± 0.19		0.11 ± 0.11		6.11 ± 0.67	19.67 ± 1.35	22.00 ± 1.90[b]
	Diabetic	6.33 ± 0.38	0.67 ± 0.19	0.22 ± 0.11				4.34 ± 0.88	11.56 ± 1.28	12.44 ± 1.42[c]
200	Non diabetic	21.89 ± 2.92	3.33 ± 0.33	1.22 ± 0.40	0.89 ± 0.29	0.33 ± 0.19		12.56 ± 1.06	40.22 ± 2.06	46.89 ± 2.61[d]
	Diabetic	12.22 ± 0.64	1.78 ± 0.24	0.56 ± 0.09		0.11 ± 0.09	0.33 ± 0.16	9.89 ± 0.79	24.89 ± 1.18	27.67 ± 1.40[e]
250	Non diabetic	35.33 ± 2.65	5.11 ± 0.40	1.00 ± 0.39	0.33 ± 0.19	0.33 ± 0.00	0.11 ± 0.11	18.56 ± 0.68	60.78 ± 3.85	68.00 ± 4.55[f]
	Diabetic	20.33 ± 1.58	2.44 ± 0.29	0.67 ± 0.19	0.45 ± 0.22	0.33 ± 0.19	0.78 ± 0.11	16.00 ± 1.45	41.00 ± 0.51	46.11 ± 0.87[d]
300	Non diabetic	48.44 ± 2.60	7.22 ± 0.73	2.44 ± 0.48	0.78 ± 0.11	0.45 ± 0.22	0.33 ± 0.19	28.22 ± 2.00	87.89 ± 0.73	99.89 ± 1.37[g]
	Diabetic	24.44 ± 1.44	4.44 ± 0.44	0.44 ± 0.29	0.56 ± 0.29	0.33 ± 0.19	0.45 ± 0.22	27.56 ± 2.35	58.22 ± 1.64	65.00 ± 1.35[f]

Note: Pooled data from three independent experiments; 900 cells were analysed per dose; 3 animals were used for each treatment. B′ – Chromatid breaks; B″ – Isochromatid breaks; RB′ – Chromatid exchange; RB′B″ – Triradials; ID – Intrachromatid deletion. Values with same superscripts are not significant ($p > 0.05$); values with different superscripts are significantly different from one another ($p < 0.05$) according to Duncan Post hoc test.

in 10 ml of 4% TCA and centrifuged at 3000 rpm for 10 min. To the supernatant, 4 ml of anthrone reagent was added. Then test tubes were cooled to room temperature and OD was measured at 620 nm against the blank.

2.6.3. Estimation of Malondialdehyde

The concentration of Malondialdehyde (MDA) was determined as lipid peroxidation index using the method of Uchiyama and Mihara [25]. Briefly, liver homogenate was mixed with trichloroacetic acid (20%) and the precipitate was dispersed by H_2SO_4 (0.05 M). Then, TBA (0.2% in 2 M sodium sulphate) was added and heated for 30 min in boiling water bath. To this, 3 ml n-butanol is added which gives pink color at the upper layer. This pink colored solution was aspirated and absorbance was measured at 532 nm.

2.7. Statistical analyses

The data were expressed as Mean ± S.E and compared using a one way analysis of variance (ANOVA). Comparisons among groups were made according to Duncun's post hoc comparison test. Significance level was $p < 0.05$.

Table 2 Percentage frequency of chromatid aberrations (Mean ± SE) induced by different doses of EMS in non diabetic and diabetic mice recovered after 24, 48 and 72 h.

Treatment in mg/kg bw.		Chromatid aberrations		
		24 h	48 h	72 h
Control I	Non diabetic	2.23 ± 0.30[a]	2.23 ± 0.30[a]	2.22 ± 0.22[a]
Control II	Diabetic	2.33 ± 0.29[a]	2.33 ± 0.29[a]	2.33 ± 0.29[a]
50	Non diabetic	2.55 ± 1.10[a]	2.22 ± 0.44[a]	2.00 ± 0.11[a]
	Diabetic	2.67 ± 1.40[a]	2.66 ± 0.40[a]	2.77 ± 0.22[a]
100	Non diabetic	17.33 ± 0.88[b]	15.33 ± 1.26[b]	10.56 ± 0.11[b]
	Diabetic	10.67 ± 0.40[c]	10.00 ± 0.88[c]	9.44 ± 0.81[b]
200	Non diabetic	34.45 ± 1.10[d]	26.55 ± 1.69[d]	18.00 ± 0.22[c]
	Diabetic	22.11 ± 2.23[e]	18.00 ± 0.77[e]	14.12 ± 0.22[d]
250	Non diabetic	53.89 ± 1.40[f]	36.67 ± 2.80[f]	24.11 ± 0.33[e]
	Diabetic	36.33 ± 0.89[d]	23.55 ± 1.92[d]	16.22 ± 0.44[f]
300	Non diabetic	76.66 ± 3.67[g]	66.00 ± 7.27[g]	40.55 ± 0.66[g]
	Diabetic	52.00 ± 2.2[f]	32.34 ± 1.95[h]	24.88 ± 0.88[e]

Note: Pooled data from three independent experiments; 900 cells were analysed per dose; 3 animals were used for each treatment. Values with same superscripts are not significant ($p > 0.05$); values with different superscripts are significantly different from one another ($p < 0.05$) according to Duncan Post hoc test.
[a–h] have been used to distinguish different values with statistical significance.

Table 3 Percentage frequency of chromatid aberrations (Mean ± SE) induced by different doses of MMS in non diabetic and diabetic mice recovered after 24, 48 and 72 h.

Treatment in mg/kg bw.		Chromatid aberrations		
		24 h	48 h	72 h
Control I	Non diabetic	2.22 ± 0.22[a]	2.22 ± 0.22[a]	2.22 ± 0.22[a]
Control II	Diabetic	2.33 ± 0.29[a]	2.44 ± 0.29[a]	2.44 ± 0.18[a]
35	Non diabetic	15.34 ± 0.95[b]	12.44 ± 0.59[b]	6.89 ± 0.22[b]
	Diabetic	11.22 ± 0.68[b]	8.56 ± 0.40[b]	6.22 ± 0.45[b]
70	Non diabetic	33.11 ± 1.11[c]	25.01 ± 0.67[c]	18.44 ± 1.25[c]
	Diabetic	23.11 ± 1.22[d]	17.89 ± 0.89[d]	18.39 ± 0.33[d]
105	Non diabetic	49.89 ± 1.66[e]	39.56 ± 3.48[e]	32.45 ± 1.07[e]
	Diabetic	37.45 ± 0.44[f]	28.33 ± 0.89[c]	21.67 ± 0.51[f]
140	Non diabetic	67.22 ± 2.45[g]	53.23 ± 1.85[f]	43.33 ± 0.89[g]
	Diabetic	49.33 ± 1.35[e]	39.22 ± 0.44[e]	20.00 ± 0.71[e]
175	Non diabetic	Animals death		
	Diabetic	88.00 ± 1.97[h]	66.44 ± 2.47[g]	52.11 ± 1.64[h]

Note: Pooled data from three independent experiments; 900 cells were analysed per dose; 3 animals were used for each treatment. Values with same superscripts are not significant ($p > 0.05$); values with different superscripts are significantly different from one another ($p < 0.05$) according to Duncan Post hoc test.

3. Results

3.1. Mortality with the treatments of EMS or MMS

Non diabetic mice treated with 400 mg/kg body weight of EMS and 175 mg/kg body weight of MMS did not survive up to 24 h, while the diabetic mice survived.

3.2. Clastogenic effect of EMS and MMS

The results of treatments with different concentrations of EMS ranging from 50 to 300 mg/kg body weight or 35–175 mg/kg body weight of MMS in diabetic and non diabetic mice with respective controls are presented in Tables 1–3. Diabetic (control II) and non diabetic (control I) mice without chemical treatment showed almost similar chromosomal aberrations. Similarly, the lowest dose of EMS employed (50 mg/kg body weight) could not produce significant chromosomal aberrations ($p > 0.05$), whereas, 100 mg/kg body weight and the above tested doses induced significant dose dependent increases in chromosomal aberrations in both categories of mice at all RTs tested ($p < 0.05$, Tables 1 and 2). Fig 1a shows a linear significant decrease in chromosomal aberrations from the first RT to last RT except the lowest dose in non diabetic mice, whereas, in diabetics (Fig 1b), only higher doses such as 200, 250 and 300 mg/kg body weight of EMS showed significant decreases in chromosomal aberrations ($p < 0.05$).

Significant dose dependent increases in the frequency of chromosomal aberrations up to 140 mg/kg body weight induced by MMS in non diabetic and diabetic mice were observed at all tested RTs compared to controls (Table 3) ($p < 0.05$). 127.33%, 91.89% and 63% chromosomal aberrations were noticed at 175 mg/kg body weight in diabetic mice at 24, 48 and 72 h RTs respectively (Table 3) and were significant compared to controls (2.22%) ($p < 0.05$). A linear significant decrease in chromosomal aberrations was noticed from 24 to 72 h RT after treatment with respective doses in both non diabetic and diabetic ($p < 0.05$, Fig 2a and b). EMS or MMS treated diabetic mice showed significant reduction in chromatid aberrations after 24, 48 and 72 h RTs compared to their respective doses in non diabetics (Table 2 and 3) ($p < 0.05$) except lower doses.

3.3. Biochemical assays

3.3.1. Catalase activity

The catalase activity is decreased in a dose dependent manner in diabetic mice treated with EMS except at 24 and 48 h RTs of

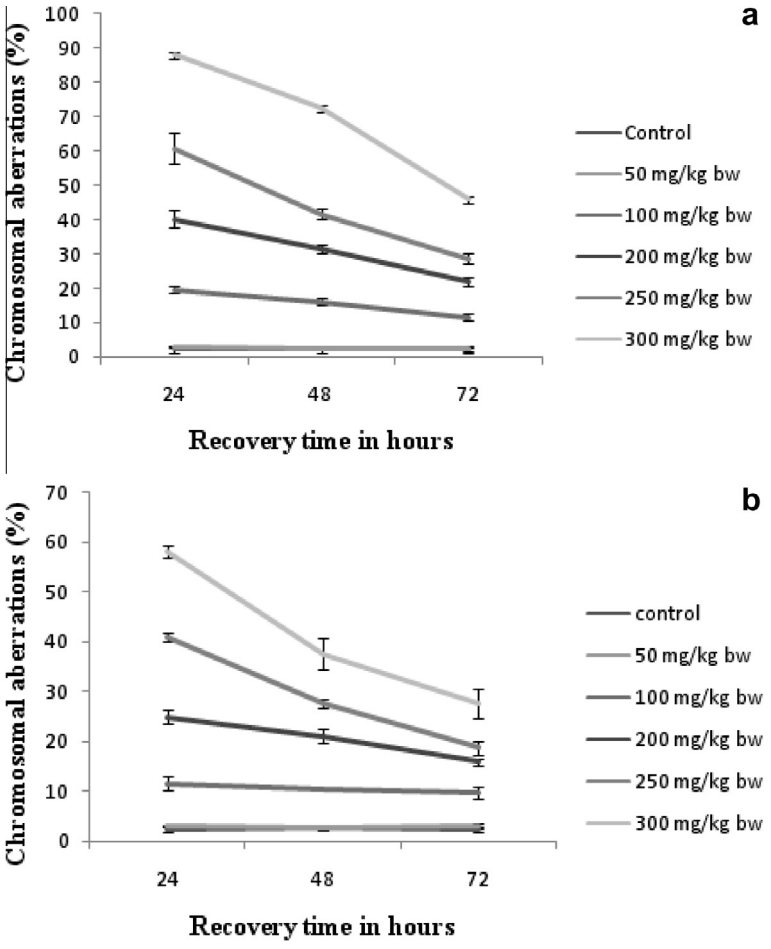

Figure 1 Percentage of chromosomal aberrations at different doses of EMS (a) non diabetic (b) diabetic mice recovered at different RTs. *Note:* Pooled data from three independent experiments; 3 animals were used for each treatment.

50 and 100 mg/kg body weight. In non diabetics the results are in contrast to the diabetic. In both diabetic and non diabetic, at 72 h RT, EMS could not significantly alter the catalase activity at different dose levels (Fig. 3a).

However in diabetic mouse, MMS caused an increase in catalase activity in a dose dependent manner at 24 h RT, except the lowest dose. In contrast, to this, in non diabetic mice, there is a decrease in activity. On the other hand at 48 and 72 h RTs there is not much variation in activity in both diabetic and non diabetic mice (Fig 3b).

3.3.2. Glycogen content

Among the controls, the non diabetic control (16.2 µg/g) showed significantly high glycogen levels compared to diabetics (10.3 µg/g). In the treatment schedules, there is significant reduction in glycogen levels at all tested doses except 50 mg/kg body weight at 72 h RTs in non diabetic and at higher doses of 200, 250, and 300 mg/kg body weight of EMS of diabetic mice at all tested RTs compared to respective controls ($p < 0.05$) (Fig 4a).

Contrary to this, no reduction in glycogen levels was seen when MMS was treated to diabetic mice at lower doses such as 35, 70 and 105 at 24 h and 35, and 70 at 48 h and all tested doses at 72 h RTs. In non diabetic mice MMS treatment significantly reduced glycogen levels at 24 h and 48 h RT at all tested doses except for 35 mg/kg body weight at 48 h RT (Fig 4b).

3.3.3. Malondialdehyde level

MDA level was significantly elevated in diabetic (control II) compared to non diabetic (control I) mice. Similarly, in the treatments also both EMS and MMS showed significant increases in MDA levels in non diabetic and diabetic mice at different RTs, except all tested doses at 72 h and the lowest dose at 24 and 48 h of diabetic mice, while in non diabetic only the lowest dose at 48 and 72 h in the case of EMS and 24 and 72 h in MMS treatments were insignificant ($p > 0.05$ Fig 5a and b).

4. Discussion

EMS and MMS induced significant chromosomal aberrations in both diabetic and non diabetic mice ($p < 0.05$) (Figs 1 and 2). These results are on par with the data of earlier workers where they have demonstrated the induction of chromosomal aberrations by employing the said agents in varied test systems and majority of aberrations were of chromatid type induced by EMS or MMS [8,26–29]. Further, dose dependent induction of

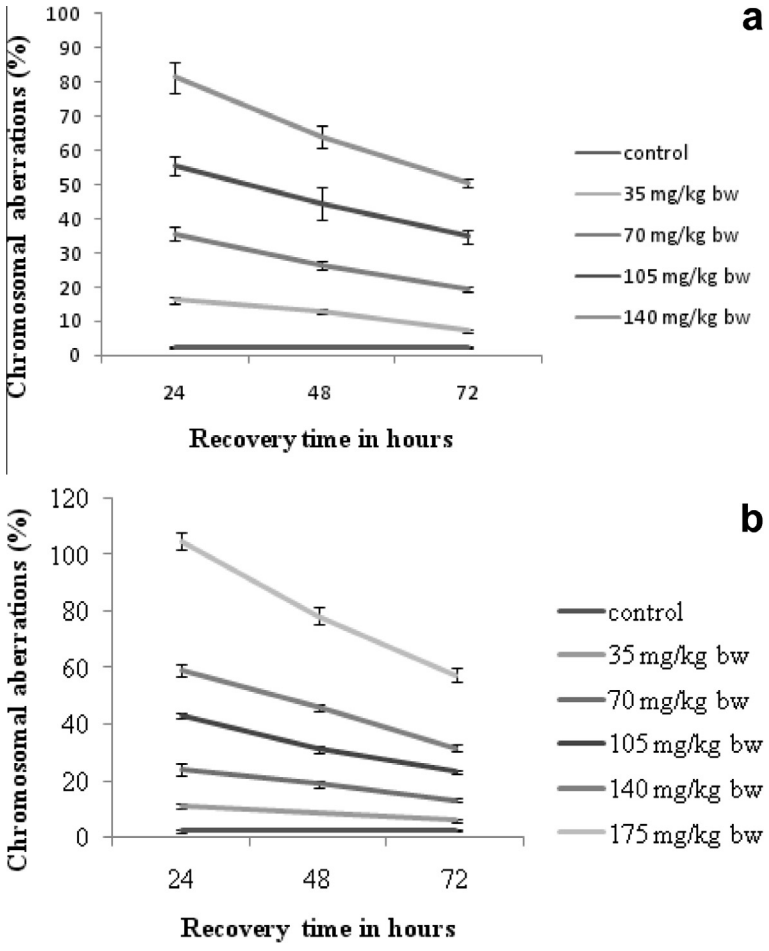

Figure 2 Percentage of chromosomal aberrations at different doses of MMS (a) non diabetic (b) diabetic mice recovered at different RTs. *Note:* Pooled data from three independent experiments; 900 cells were analysed per dose; 3 animals were used for each treatment.

aberrations was noticed in the present investigation in diabetic and non diabetic mice as has been observed by earlier workers [27,30]. Even though, chromatid aberrations were produced in diabetic and non diabetic mice, it is interesting to note that the percentage of chromatid aberrations was less in diabetic than non diabetic mice. For example 300 mg/kg body weight of EMS induced 52% of chromatid aberrations in diabetic mice and 76% in non diabetic and 140 mg/kg body weight of MMS induced 49% of chromatid aberrations in diabetic and 67% in non diabetic mice. As far our knowledge goes, it is the first report on the induction of chromatid aberrations in diabetic mice in general and significantly less than non diabetic in particular. Thus, in the present study diabetic mice showed resistance to EMS or MMS induced chromosomal aberrations. This type of resistance of the diabetic mouse to alkylating agents can be viewed from the hypothesis that short term starvation (48–60 h) or low glucose can protect mammalian cells against damaging effects of high doses of chemotherapy. Raffaghello et al. [31] tested the effect of normal glucose (1.0 g/L), low glucose (0.5 g/L) and high glucose (3.0 g/L) on H_2O_2 treatment in rat glial cells. The results have revealed that 80% of rat glial cells pretreated with normal and low glucose were resistant, while only 10% of cells pretreated with high glucose survived. However, cytotoxicity of H_2O_2 toward cancer cells was unaffected. In the similar experiment, 80% of glial cells were resistant to 12 mg/ml cyclophosphamide (CP-widely used in cancer treatment) in the presence of 0.5 g/lt glucose, whereas only 20% of the cells survived this treatment in 1 g/lt glucose [31]. When another widely used chemotherapeutic drug namely etoposide (damage DNA and cause toxicity) was used, 80 mg/kg killed 43% of control mice by day 10; but only 1% of the mice that were pre-starved died after etoposide treatment [31]. Thus, it was concluded that short term starvation greatly improves early survival by ameliorating chemotherapy toxicity. When yeast cells were cultured in low glucose media and treated with MMS, they showed increased survival rate [32], demonstrating that low glucose has reduced glycolytic rate, resulting in repression of respiration and TCA enzymes, and increased citrate synthase activity, as a consequence pyruvate is utilized more effectively in mitochondria, enabling NADH production in TCA and NAD^+ regeneration, which could serve as a protective mechanism against MMS, enabling more cells to survive. In the case of *Escherichia coli*, prior to MMS treatment cells cultured in 0.2% and 0.4% of glucose showed an increased survival rate compared to cells cultured in normal media [33] and, termed it as "Glucose induced resistance (GIR)", proposing that GIR is a consequence of enhanced repair capability involving

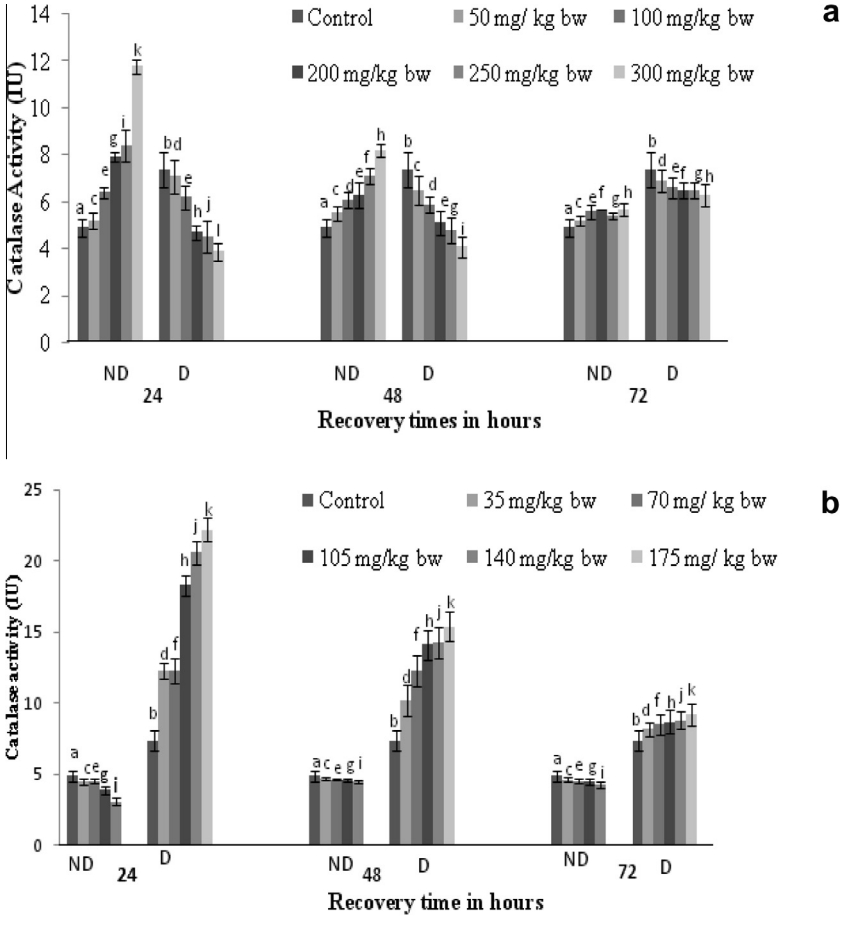

Figure 3 Catalase activity in the liver of mice at different RTs (a) EMS (b) MMS. *Note:* Pooled data from three independent experiments; 3 animals were used for each treatment. ND – Non diabetic, D – Diabetic. Statistical analysis was done using Duncan Post hoc test between diabetic and non diabetic groups, values with different superscripts are significantly different from one another ($p < 0.05$).

at least some of the gene products of the Rec system. Bjorkhem [34] has studied the inhibition of ω oxidation of long chain fatty acid in the normal, starved and diabetic rat liver and concluded that ω oxidation is inhibited in normal rat liver cells where as in diabetic and starvation state this inhibition is released. Thus they concluded that the physiological condition of both diabetic and starved state is utmost important. In the case of starvation cells depend upon an endogenous source and undergo gluconeogenesis for energy [35,36]. Even under diabetic conditions, cells depend upon endogenous sources by accelerating gluconeogenesis and a decrease in glycolysis [*cf.* 37], but these cells cannot utilize the external glucose for energy [38]. With these lines of evidence, it can be opined that the physiological condition of both starved and diabetic cells are the same and thus in the present study decreased chromatid aberrations in EMS or MMS treated diabetic mice might be because of altered metabolic pathways as of starved cells.

The results of the present study show that a relatively low dose of MMS is sufficient to induce significant chromosomal aberrations in comparison to EMS in diabetic and non diabetic mice. This points out that MMS is a more potent inducer of chromosomal aberrations in diabetic and non diabetic mice. Similarly, Riaz Mahmood [27] working on normal (non diabetic) mitotic cell of mouse; Sega and Owens [39] on normal mouse germ cells; Rao and Natarajan [40] using *Vicia faba*; Siddique et al. [5] in *Drosophila*; Riaz Mahmood and Vasudev [10] in *P. pictus*; Vogel and Natarajan [cf. 26] in CHO cells and Harish et al. [17] employing Human lymphocytes have demonstrated the potency of MMS to induce more chromosomal aberrations than EMS. The postulated reasons can be that (1) EMS is an ethylating agent and MMS is a methylating agent and thus methylating agent may be a potent inducer of chromosomal aberrations in mice than ethylating agent. (2) Swain scott constant (*s*) value of MMS is high (0.83) compared to EMS (0.67). Using the same equation, in *in vivo* systems such as *V. faba* and *Drosophila*, Rao and Natarajan [40] and Vogel and Natarajan [41] respectively have demonstrated that alkylating agents with high s-value were found to be more cytotoxic and less mutagenic than with low *s* value. Thus present results also underline the importance of *s* value in the induction of chromosomal aberrations whether diabetic or non diabetic mice.

There is reduction in the frequency of aberrations from 24 to 72 h RTs with EMS or MMS in diabetic and non diabetic mice. This is on par with Obe and Beek [42], where they observed a decreased aberration frequency with an increase in culture time in human lymphocytes reflecting a mechanism of 'mitotic selection' of aberration bearing cells. Similar observations in non diabetic mice were also made by Riaz Mahmood [27].

Working on rat liver catalase activity under the influence of chlorfenvinphos (CVP), an organophosphate insecticide, Lukaszewich-Hussain and Moniusko-Jakoniuk [43] showed the increased activity at 1st h at the dose of 0.2 LD_{50} and 1st and 24 h at the dose of 0.1 LD_{50}. Similarly, in the present study in non diabetic mice, the activity of catalase tended to increase

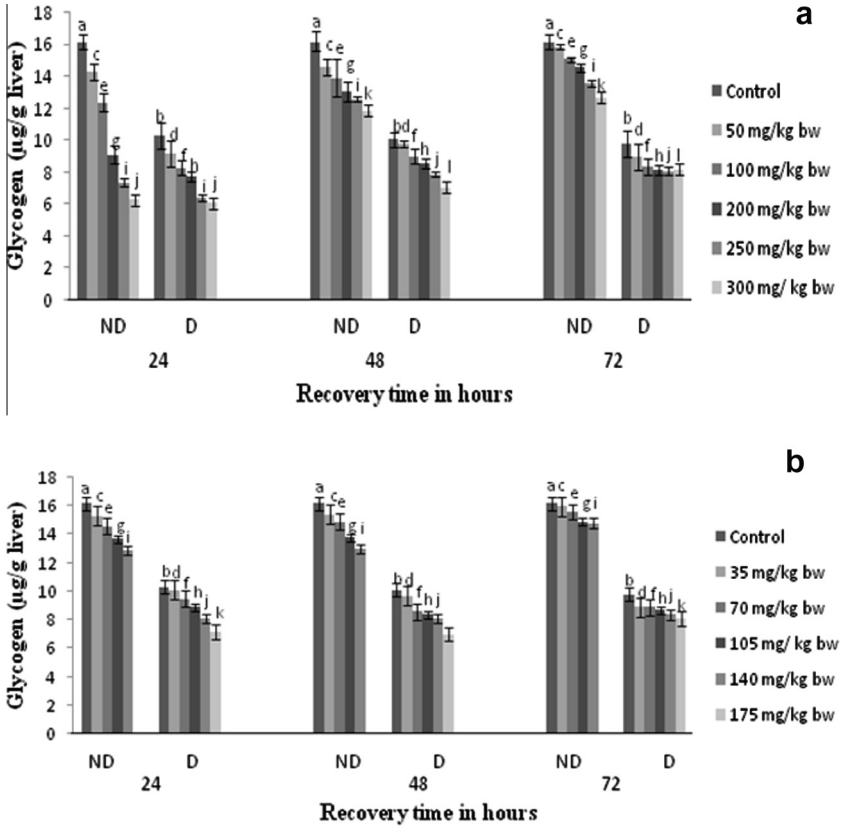

Figure 4 Glycogen levels in the liver of mice at different RTs (a) EMS (b) MMS. *Note:* Pooled data from three independent experiments; 3 animals were used for each treatment. ND – Non diabetic, D – Diabetic. Stastical analysis was done using Duncan Post hoc test between diabetic and non diabetic groups, values with different superscripts are significantly different from one another ($p < 0.05$).

with increase in EMS dose at 24 and 48 h RTs. Contrary to this, the results were vice versa in diabetic mice. In the case of MMS treatment catalase activity increased in diabetic mice and decreased in non diabetic at 24 h RTs. However, with EMS at 72 h RT and MMS at 48 and 72 h in both diabetic and non diabetic mice with different doses, the activity of catalase did not exhibit significant changes, thus, it can be opined that the changes in the activity of catalase were not dose dependent. Working in non diabetic mice Guruprasad et al. [44] have demonstrated an increase in catalase activity with EMS and decreased activity with MMS treatment which is on par with our studies in non diabetic mice. Salmon et al. [45] and Friedberg et al. [46] have showed that MMS cannot produce ROS directly, but induced the DNA damage causing an increase in intracellular ROS level in yeast cells. Cyclophosphamide treatment has shown decreased catalase activity in the liver and lung of mice [47] and with Chlorambucil in the rat liver [48]. When HL-60 cells were treated with alkylating agents such as melphalan and Chlorambucil an increase in intracellular peroxide was reported. This increase in peroxide was time and concentration dependent that induced apoptosis. Pre treatment of HL-60 cells with catalase provided partial protection against low concentration of melphalan, whereas Chlorambucil could not induce protection against apoptosis, suggesting that hydrogen peroxide is not the main peroxide produced by Chlorambucil treatment [49]. At this juncture it is pertinent to mention that there are few data concerning the influence of alkylating agents on the activity of catalase and for this reason it is difficult to compare our results with those reported by other authors. The interpretation of the decrease and increase in the catalase activity is difficult as has been discussed by Lukaszewich-Hussain and Moniusko-Jakoniuk [43], even though, the increase in activity can be explained as the response of the liver to high levels of H_2O_2. The decreased activity of catalase may be because of the inhibition of catalase activity by superoxide anion as has been proposed by Laszlo et al. [50].

Glycogen is the polymer of glucose which is known as animal starch and it is the most readily available source of energy in the animal tissues. In the present studies both diabetic and non diabetic mice have a decreased glycogen content. However, it is befitting to note that between diabetic and non diabetic, a significant decrease is observed in treated diabetic mice. Mancozeb and carbofuran pesticides have increased the level of glycogen [51,52]. Carbosulfan caused decreased glycogen with increasing dose exposure [53]. Treatment with diethylnitrosamine (DENA) and dimethylnitrosoamine (DMNA) [54] has reduced liver glycogen in the rat. Dermal application of sulfur mustard has reduced the glycogen content in the liver [55]. In our study a greater

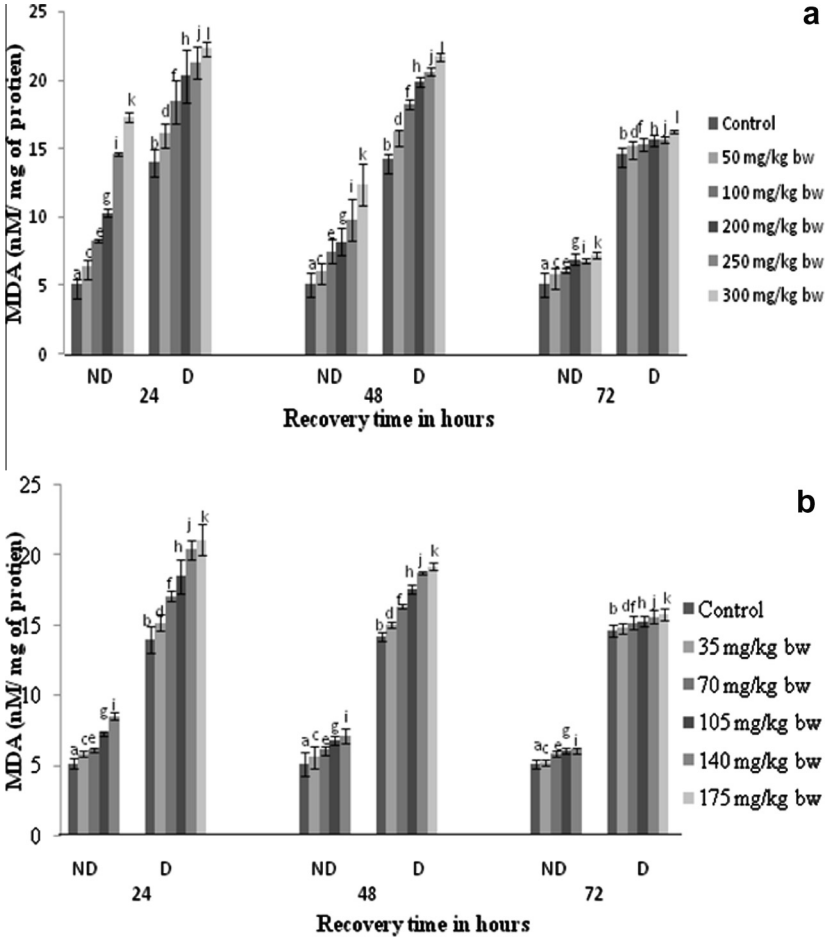

Figure 5 MDA levels in the liver of mice at different RTs (a) EMS (b) MMS. *Note:* Pooled data from three independent experiments; 3 animals were used for each treatment. ND – Non diabetic, D – Diabetic. Stastical analysis was done using Duncan Post hoc test between diabetic and non diabetic groups, values with different superscripts are significantly different from one another ($p < 0.05$).

reduction in liver glycogen was observed in EMS than in MMS in both diabetic and non diabetic mice. Decreased levels of glycogen may be due to either an increased catabolism of the biomolecules to meet the enhanced energy demand of animals under stress or their reduced synthesis due to impaired tissue function [56].

When antioxidants are no longer capable of coping with ROS, results in oxidative stress in turn affect cellular integrity. This ROS reacts with the unsaturated fatty acid of cellular or sub cellular membranes and they lead to peroxidation of membrane lipids. The oxidative stress caused by different agents is often estimated by the level of MDA [57]. Diabetic mice showed increased lipid peroxidation compared to non diabetic and the reason might be due the variation in lipid metabolism [58] which alters the function of cellular membrane, inhibiting the activity of superoxide dismutase enzyme leading to accumulation of superoxide radicals resulting in maximum lipid peroxidation and tissue damage in diabetes [59], also increased glycation of protein in diabetes mellitus. The glycated protein might themselves act as a source of free radicals. There is a clear association between lipid peroxide and glucose concentration, which may be also thought to play a role in increased lipid peroxidation in diabetes mellitus [60]. The results after the treatment with EMS or MMS in both diabetic and non diabetics showed enhanced levels of MDA at 24 h RT. Further it is interesting to note that level of MDA decreased with increasing time. Lukaszewich-Hussain and Moniusko-Jakoniuk [43] demonstrated the increased MDA level at 48th h after intoxication with CVP at a dose of 0.5 LD_{50} and as early as at 1 h after intoxication with two lower doses of insecticide. At 24 h of intoxication with insecticide of 0.5 LD_{50}, a decreased MDA level was observed. This along with our results, indicate that the prooxidant effect of the highest dose of agents becomes significant only after later time of intoxication, while the same effect of lower doses is evident much earlier. Eventhough, there are no reports on the effect of alkylating agents on MDA levels, the increased level of MDA in intoxication with organophosphate insecticide has been reported [61,62]. A dose-dependent increase in MDA concentration was found in the renal tubules of rats after treatment with acephate [63]. The MDA concentration was increased also in the skeletal muscles of rats intoxicated with diisopropylfluorophosphate [50]. Treatment with Chorambucil induced the lipid peroxidation in rat liver [48]. In mice, lipid peroxidation was increased in the liver and lung treated with Cyclophosphamide [47]. In an *in vitro* study, Cisplatin treatment has increased the lipid peroxidation in human erythrocytes [64].

In conclusion it can be stated that (1) tested alkylating agents EMS and MMS induced chromatid type of aberrations in diabetic mice in a dose dependent manner, (2) methylating agent (MMS) was a more potent inducer of chromatid type aberrations than ethylating (EMS), (3) diabetic mouse is more resistant than non diabetic to alkylating agents and (4) the tested agents altered the analyzed biochemical parameters such as catalase, glycogen and MDA.

Duality of interest

The authors declare that there is no duality of interest associated with this manuscript.

Acknowledgements

The authors wish to express their gratitude to the Director of Post-Graduate Centre, University of Mysore, Hemagangothri, Hassan, for providing facilities and also thank Professor B.S. Vishwanath (Department of Biochemistry) and Professor H.N. Yajurvedi (Department of Zoology), University of Mysore, Mysore, for their kind help during the course of this work.

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