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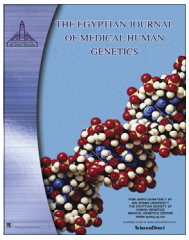

ORIGINAL ARTICLE

Inducible protective processes in animal systems XIV: Cytogenetic adaptive response induced by EMS or MMS in bone marrow cells of diabetic mouse

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KEYWORDS

EMS;
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Diabetic mouse;
Conditioning dose;
Challenging dose;
Combined dose

Abstract *Background:* Adaptive response has been well studied by employing physical and chemical agents in normal test systems, whereas in diseased conditions very little data are available.

Aim of the study: To know the presence or absence of adaptive response in diseased condition, alkylating agents such as EMS or MMS have been employed in diabetic mouse.

Material and methods: To induce diabetes, mice were injected with 180 mg/kg body weight of Stz. Diabetic mice were treated with conditioning (100 mg/kg body weight of EMS or 40 mg/kg body weight of MMS), challenging (300 mg/kg body weight of EMS or 160 mg/kg body weight of MMS) and combined doses of EMS or MMS with 8 h time lag. Parallelly controls were maintained. Mice were sacrificed at 24 or 48 or 72 h RTs. Bone marrow was extracted and slides were prepared by a routine air dry technique by Evans et al. (1964) to analyze the chromosomal aberrations.

Results: The results show that both the alkylating agents induced exclusively chromatid type of aberrations in both diabetic and non diabetic mice, but it is to be underlined that MMS is a more potent inducer of aberrations than EMS. Eventhough, combined treatment of EMS or MMS induced significantly less chromosomal breaks compared to challenging treatment ($p < 0.05$) in diabetic mice, EMS induced 40% reduction of breaks, compared to 51.74% by MMS at 24 h RT. This is true to other tested RTs.

Conclusion: (1) Methylating agents are a more effective inducer of adaptive response than ethylating agents in diabetic mouse. (2) Further, it is interesting to note that the percentage reduction of chromosomal breaks in diabetics is comparatively much less than in non diabetic mouse, inferring that there is variation in adaptive response between diseased and non diseased condition.

Abbreviations: EMS, ethyl methanesulfonate; MMS, methyl methanesulfonate; Stz, Streptozotocin; RTs, recovery times

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1. Introduction

DNA is being continuously exposed to endogenous and exogenous agents and each cell receives about thousands of DNA lesions per day [1]. These lesions, commonly include base modifications, single and double strand breaks, DNA–protein cross links, and base free sites [2], if not repaired, are proved to be lethal to the cells. To overcome this, cells are well equipped with different repair mechanisms such as photoreactivation [3], excision repair [2], mismatch repair [4], homologous recombination [5], non homologous end joining [6] and inducible repair mechanisms [7]. One such inducible repair mechanism is SOS, an error prone repair mechanism [8] and the other is adaptive response, an error free repair mechanism [9]. Samson and Cairns, showed that *Escherichia coli* cells were resistant to killing and mutagenic effect of toxic treatment of *N*-methyl, *N*-nitro-*N*-nitrosoguanidine (MNNG), which were pre treated with a sub toxic dose of the same agent. Later extensive work has been carried out in both prokaryotes and eukaryotes using physical [*cf*. 10] and chemical [*cf*. 11] agents. Mahadimane and Vasudev [12] reported the presence of adaptive response in *in vivo* ehrlich ascites tumor cells, a cancerous condition. However, adaptive response has not been worked out in various diseased conditions such as Alzheimer, arthritis, asthma, bronchitis and diabetes, which require immediate attention. Diabetes is a multifarious disease and 7th leading cause of death around the world. About 387 million people are suffering from diabetes around the world and expected to reach 592 million by the end of 2035 (International Diabetic Federation, 2014). These diabetic patients are exposed to varied chemical agents/drugs, which might lead to serious disorders. In our previous study, it has been demonstrated that EMS and MMS induced less number of chromosomal aberrations in diabetic compared to non diabetic mice [13]. As has been said, there is increasing trend of diabetic patients around the world, who are being exposed to various chemicals including alkylating agents. There is a necessity to get the picture of adaptive response in diabetic systems. In order to know the presence or absence of adaptive response in diabetic condition, the present investigation has been carried out by employing EMS or MMS as alkylating agents.

2. Materials and methods

Mono functional alkylating agents, methyl methanesulfonate (MMS) (CAS number 66-27-3), and ethyl methanesulfonate (EMS) (CAS number 62-50-0) were obtained from Sigma Co., St. Louis, MO, USA, and colchicine (CAS number 64-86-8) was obtained from Himedia, Pvt. Ltd., Mumbai, India. Giemsa stain and other chemicals of analytical grade were commercially available. EMS and MMS were dissolved in 0.9% NaCl to obtain the required concentrations. Freshly prepared solutions of these agents were used each time. From the dose effect relationship study by Khalandar and Vasudev [13], a dose which induced few chromosomal aberrations and a dose that produced high aberrations were selected as conditioning and challenging doses respectively. Accordingly for EMS 100 mg/kg body weight as the conditioning and 300 mg/kg body weight as challenging dose and for MMS 40 mg/kg body weight and 160 mg/kg body weight as conditioning and challenging dose were selected respectively.

2.1. Animals

Male Swiss albino mice weighing 25–30 g of 6–8 weeks old were used and housed in polypropylene cages, provided with standard feed pellets and water *ad libitum* under 12 h of 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 (Declaration of Helsinki) for experiments in animals.

2.2. Induction of diabetes in mouse

Diabetes was induced by injecting a single intra peritoneal dose of Streptozotocin (180 mg/kg body weight) (freshly prepared in 0.1 M citrate buffer pH 4.5) as described by Yanardag et al. [14]. 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 [15].

2.3. Treatment schedule

Diabetic and non diabetic mice were grouped from A to D for MMS and EMS, each group consisting of 3 animals.

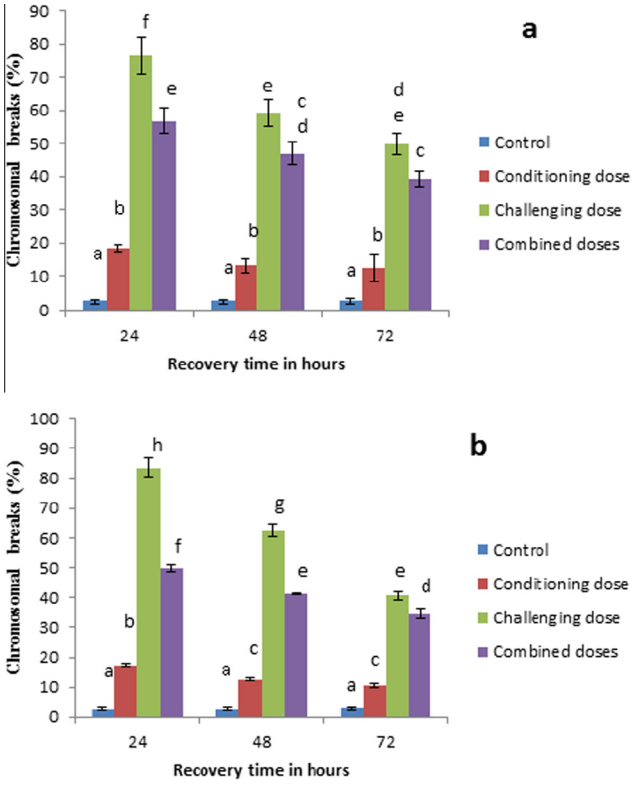

Figure 1 Yield of chromosomal breaks induced after different treatment schedules of EMS and (b) MMS. *Note:* values with different superscripts are significantly different from one another ($p < 0.05$) according to Duncan Post hoc test.

Diabetic and non diabetic mice were injected with 0.5 ml saline containing conditioning dose of 100 or 40 mg/kg body weight and challenging dose of 300 or 160 mg/kg body weight of EMS or MMS respectively. For adaptive response the animals were injected with conditioning dose of EMS (100 mg/kg body weight) or MMS (40 mg/kg body weight) and after 8 h animals were injected with respective challenging dose (EMS-300 mg/kg body weight and MMS-160 mg/kg body weight). The control animals received only 0.5 ml of saline. Animals were sacrificed at 24, 48 and 72 h recovery times (RTs).

2.4. Mitotic chromosome preparation

90 min before killing, the treated animals were injected intraperitoneally with 0.5 ml of 0.05% colchicine. Bone marrow was extracted and slides were prepared by a routine air dry technique [16]. 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, intrachromatid deletions, triradials, chromosome breaks, dicentrics, rings, and minutes. 100 well spread metaphase plates were scored for each animal and experiments were repeated thrice.

2.5. 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.

3. Results

The conditioning dose of EMS or MMS resulted in significant aberrations and on the other hand, maximum number of aberrations were noticed when challenging dose of said agents was given to diabetic mouse ($p < 0.05$) compared to controls. The same results were observed at all RTs tested (Tables 1–3). 61.56% of chromatid aberrations which were the majority, was observed when the challenging dose of MMS was given to diabetic mouse compared to 11.11% chromosome aberrations at 24 h RT. The same is true for other treatments and other RTs tested (Tables 2–5). However, when the conditioning and challenging dose were given together (combined treatment) to diabetic mouse, the results have shown that there is significant reduction in the number of total chromosomal aberrations at all RTs of the tested agents (Tables 1–5) compared to challenging dose ($p < 0.05$).

When the chromosomal aberrations were converted to chromosomal breaks and when individual treatments were contrasted, the results have revealed that challenging dose of EMS in diabetic mouse induced 76.55 ± 3.58% breaks whereas the combined dose produced 56.77 ± 3.87% breaks at 24 h RT, which is significant ($p < 0.05$) (Fig. 1(a)). The same is observed at different RTs and treatments (Fig. 1 (a) and (b)). Tables 6 and 7 gives the percentage reduction of chromosomal breaks in EMS or MMS treated diabetic mouse at different RTs tested compared to additive effects.

Table 1 Percentage frequency of chromosomal aberrations (mean ± SE) induced by conditioning, challenging, and combined doses of EMS in non diabetic and diabetic mice bone marrow cells recovered after 24 h.

Treatments		B′	B″	RB′	RB′B″	ID	Ring	Minutes	Total aberration
Control	Non diabetic	1.67 ± 0.19						0.56 ± 0.11	2.22 ± 0.22[a]
	Diabetic	1.22 ± 0.22	0.11 ± 0.11					1.11 ± 0.22	2.44 ± 0.40[a]
Conditioning	Non diabetic	20.18 ± 0.76	1.89 ± 0.22	1.00 ± 0.51		0.11 ± 0.11		6.56 ± 0.95	29.74 ± 0.80[b]
	Diabetic	10.44 ± 0.29	1.00 ± 0.27	0.67 ± 0.27				5.78 ± 0.11	17.33 ± 0.38[b]
Challenging	Non diabetic	54.00 ± 6.56	7.11 ± 0.95	2.00 ± 0.55	0.78 ± 0.11	0.33 ± 0.78	1.78 ± 0.45	27.78 ± 3.01	93.78 ± 10.48[e]
	Diabetic	24.11 ± 1.25	4.67 ± 0.96	0.55 ± 0.22	0.67 ± 0.19	0.78 ± 0.29	1.22 ± 0.4	37.22 ± 3.01	69.22 ± 5.00[d]
Combined	Non diabetic	30.33 ± 1.20	4.33 ± 0.88	1.33 ± 0.33	0.84 ± 0.13	0.33 ± 0.33	0.84 ± 0.13	26.00 ± 2.65	63.45 ± 3.28[cd]
	Diabetic	21.33 ± 2.40	2.44 ± 0.80	0.89 ± 0.44	0.44 ± 0.29		2.22 ± 0.22	23.00 ± 1.00	50.33 ± 3.46[c]

Note: Pooled data from three independent experiments; 900 cells were analyzed 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.

4. Discussion

DNA is the target for all alkylating agents which induce lesions that in turn result in micronucleus [17,18], sister chromatid exchanges [19,20], intra chromatid deletions, rings, iso chromatid breaks and chromatid breaks [*cf*. [21]] in varied test systems. EMS and MMS, the monofunctional alkylating agents, used in the present investigation induced a more chromatid type of aberrations than chromosomal aberrations in both diabetic and non diabetic mouse system (Tables 1–5). Thus, proving that, these agents are S-dependent agents in diseased condition also. These observations are in agreement with earlier findings of Rao and Natarajan in *Vacia faba* [22], Vogel and Natarajan in *Drosophila* [23], Mahmood in non diabetic mouse [21], Harish et al. in human lymphocytes [24] and Mahadimane and Vasudev in *in vivo* ascites cells [12].

Chromosomal aberrations' study revealed that 35 mg/kg body weight of MMS is sufficient to induce 16.11% of chromosomal aberration, whereas, 100 mg/kg body weight of EMS induced almost similar number 17.33% aberrations, which is very high compared to the dose of MMS (Tables 1 and 3). For this reason it can be said that MMS is a strong inducer of chromosomal aberrations compared to EMS. Similarly Rao and Natarajan using *Vicia faba* [21], Vogel and Natarajan using *Drosophila* [22], Mahmood et al. using mouse [25], and Harish et al. using human lymphocytes [24] have shown that methylating agents are more potent inducer of aberrations than ethylating agents.

Since the first evidence of the presence of adaptive response in *E. coli* by Samson and Cairns [9] using MNNG, extensive research has been carried out by a number of scientists [10,26–30] in different test systems and generalized the discovery that pre treatment with conditioning dose protects DNA damages induced by subsequent challenge dose.

Eventhough, using the said agents, adaptive response has been well documented in varied test systems employing various end points in normal cells [21,24,12]. To the best of our knowledge there are no data on adaptive response in diseased conditions such as diabetes. To get the diabetic conditioned animals in the present investigations, mouse was injected with Streptozotocin and to analyze adaptive response the prerequisite is the selection of doses namely conditioning and challenging. These doses of EMS or MMS were selected based on the work of Khalandar and Vasudev [13] and the results have revealed that both these agents induced significant chromosomal aberrations in diabetic mouse (Tables 1–5). These findings further validate the results of earlier works [12] *cf*. [31]]. Mahmood et al. [25] demonstrated that 8 h time lag between conditioning and challenging treatments commensurate the peak level of repair enzymes. The same time lag used in the present investigations revealed the significant decrease in chromosomal aberrations with EMS or MMS conditioning (Tables 1–5). On par with this Mahadimane and Vasudev [12] with the above said preconditioning with EMS or MMS, demonstrated the presence of adaptive response using the same end point in *in vivo* ascite tumor cells. Similarly, reduction in frequency of micronuclei was reported by Assadi et al. [32], when human lymphocytes were irradiated with conditioning dose (5 cGy) before challenging dose (2 Gy) of gamma radiations compared to challenging alone. Further the same phenomenon was studied in *Drosophila* [33], *Poecilocerus pictus* [29] and Chinese hamster cells [26].

There is a strong evidence to prove that methylating agents are a more effective inducer of adaptive response than ethylating agents in normal cells [34,35,25,36]. Our results also prove the same in that, MMS reduced the chromosomal breaks to 51.74% compared to 40% with EMS in diabetic mouse at 24 h RT. Similar results were observed at tested RTs (Tables 1–5).

Table 2 Percentage frequency of chromosomal aberrations (mean ± SE) induced by conditioning, challenging and combined doses of EMS in non diabetic and diabetic mice bone marrow cells recovered after 48 h and 72 h.

Recovery time in h	Treatments		Chromosomal aberrations		Total aberrations
			Chromatid aberrations	Chromosome aberrations	
	Control	Non diabetic	2.22 ± 0.31	0	2.22 ± 0.31[a]
		Diabetic	2.33 ± 0.29	0.11 ± 0.11	2.44 ± 0.4[a]
48	Conditioning	Non diabetic	18.33 ± 1.57	0.55 ± 0.40	18.89 ± 0.80[b]
		Diabetic	11.00 ± 1.22	1.11 ± 0.63	12.11 ± 1.5[b]
	Challenging	Non diabetic	70.67 ± 6.25	7.22 ± 1.56	77.89 ± 5.79[e]
		Diabetic	46.67 ± 3.96	6.56 ± 1.43	53.23 ± 3.37[c]
	Combined	Non diabetic	53.33 ± 3.86	4.45 ± 1.78	57.78 ± 2.48[d]
		Diabetic	36.67 ± 3.67	5.00 ± 5.0	41.66 ± 3.18[f]
72	Control	Non diabetic	2.22 ± 0.31	0	2.22 ± 0.31[a]
		Diabetic	2.56 ± 0.44	0.11 ± 0.11	2.67 ± 0.51[a]
	Conditioning	Non diabetic	15.78 ± 1.46	0.55 ± 0.4	16.33 ± 0.19[b]
		Diabetic	10.33 ± 2.08	1.00 ± 0.67	11.33 ± 2.60[b]
	Challenging	Non diabetic	58.67 ± 6.41	7.11 ± 1.8	65.78 ± 4.56[e]
		Diabetic	42.00 ± 2.92	4.34 ± 0.63	46.33 ± 3.06[c]
	Combined	Non diabetic	45.33 ± 2.1	3.78 ± 1.84	49.11 ± 2.99[c]
		Diabetic	32.00 ± 2.65	3.33 ± 1.61	35.33 ± 2.08[d]

Note: pooled data from three independent experiments; 900 cells were analyzed per treatment; 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–f have been used to distinguish different values with statistical significance.

Table 3 Percentage frequency of chromosomal aberrations (mean ± SE) induced by conditioning, challenging and combined doses of MMS in non diabetic and diabetic mice bone marrow cells recovered after 24 h.

Treatments		Chromosomal aberrations		Total aberrations
		Chromatid aberrations	Chromosome aberrations	
Control	Non diabetic	2.23 ± 0.30	0	2.22 ± 0.22[a]
	Diabetic	2.33 ± 0.29	0.11 ± 0.11	2.45 ± 0.4[a]
Conditioning	Non diabetic	20.78 ± 2.37	1.22 ± 0.40	22.00 ± 2.6[b]
	Diabetic	14.89 ± 1.73	1.22 ± 0.40	16.11 ± 0.67[b]
Challenging	Non diabetic	87.45 ± 3.12	16.45 ± 2.68	103.89 ± 2.61[c]
	Diabetic	61.56 ± 3.53	11.11 ± 2.74	72.67 ± 2.00[d]
Combined	Non diabetic	46.67 ± 2.41	6.22 ± 2.75	52.89 ± 3.56[e]
	Diabetic	41.33 ± 2.40	4.11 ± 1.44	45.44 ± 1.82[f]

Note: pooled data from three independent experiments; 900 cells were analyzed 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.

Table 4 Percentage frequency of chromosomal aberrations (mean ± SE) induced by conditioning, challenging and combined doses of MMS in non diabetic and diabetic mice bone marrow cells recovered after 48 h.

Treatments		Chromosomal aberrations		Total aberrations
		Chromatid aberrations	Chromosomal aberrations	
Control	Non diabetic	2.22 ± 0.31	0	2.22 ± 0.31[a]
	Diabetic	2.33 ± 0.29	0.11 ± 0.11	2.44 ± 0.4[a]
Conditioning	Non diabetic	17.00 ± 1.35	1.00 ± 0.58	18.00 ± 0.96[b]
	Diabetic	11.00 ± 1.69	0.78 ± 0.11	11.78 ± 0.59[ab]
Challenging	Non diabetic	50.89 ± 2.78	10.00 ± 2.83	71.33 ± 9.51[c]
	Diabetic	44.33 ± 3.29	8.56 ± 2.20	52.89 ± 1.46[d]
Combined	Non diabetic	36.00 ± 2.65	4.33 ± 1.82	40.33 ± 0.67[fe]
	Diabetic	34.33 ± 2.98	3.44 ± 1.44	37.78 ± 1.22[e]

Note: pooled data from three independent experiments; 900 cells were analyzed 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.

Table 5 Percentage frequency of chromosomal aberrations (mean ± SE) induced by conditioning, challenging and combined doses of MMS in non diabetic and diabetic mice bone marrow cells recovered after 72 h.

Treatments		Chromosomal aberrations		Total number of aberrations
		Chromatid aberrations	Chromosomal aberrations	
Control	Non diabetic	2.22 ± 0.31	0	2.22 ± 0.31[a]
	Diabetic	2.33 ± 0.44	0.11 ± 0.11	2.44 ± 0.4[a]
Conditioning	Non diabetic	13.44 ± 1.13	0.67 ± 0.33	14.11 ± 1.06[b]
	Diabetic	9.66 ± 1.36	0.44 ± 0.29	10.11 ± 0.78[b]
Challenging	Non diabetic	39.22 ± 2.86	9.56 ± 1.75	48.11 ± 1.55[d]
	Diabetic	31.67 ± 3.17	4.11 ± 2.26	35.78 ± 0.59[e]
Combined	Non diabetic	32.00 ± 2.85	3.67 ± 1.91	35.67 ± 1.86[e]
	Diabetic	27.00 ± 2.22	3.44 ± 1.74	30.44 ± 0.78[f]

Note: pooled data from three independent experiments; 900 cells were analyzed per treatment; 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.

Olsson and Lindahl [34] demonstrated that *ada* coded methyltransferase, transfers the ethyl group of O6-ethylguanine at a rate of 10 times less efficient than that for the methyl transfer. In wild-type *E. coli*, the adaptive response began to contribute to O6-methylguanine repair about one hour after alkylation, which is the time required for full induction of the *ada* DNA

Table 6 Percentage reduction of chromosomal breaks (mean ± SE) after combined treatment of EMS at different recovery times in bone marrow cells of non diabetic and diabetic mouse.

RT in hours	EMS	Additive effect *A*	Combined effect *B*	Reduction of chromosomal breaks	Reduction in (%) *C*
24	Non diabetic	1.39 ± 0.11	0.71 ± 0.04	0.68 ± 0.07	49.04 ± 1.19
	Diabetic	0.95 ± 0.05	0.57 ± 0.04	0.38 ± 0.07	40.00 ± 6.07
48	Non diabetic	1.05 ± 0.08	0.62 ± 0.02	0.43 ± 0.07	40.82 ± 4.13
	Diabetic	0.73 ± 0.04	0.47 ± 0.03	0.26 ± 0.02	35.01 ± 2.47
72	Non diabetic	0.90 ± 0.06	0.53 ± 0.04	0.37 ± 0.02	41.33 ± 1.28
	Diabetic	0.62 ± 0.05	0.39 ± 0.02	0.23 ± 0.03	36.90 ± 1.57

Note: percentage of reduction was calculated using formula $C = 100 - (B/A * 100)$.

Table 7 Reduction (%) of chromosomal breaks (mean ± SE) after combined treatment of MMS at different recovery times in bone marrow cells of non diabetic and diabetic mouse.

RT in hours	MMS	Additive effect *A*	Combined effect *B*	Reduction of chromosomal breaks	Reduction in (%) *C*
24	Non diabetic	1.46 ± 0.06	0.59 ± 0.03	0.87 ± 0.03	59.59 ± 0.63
	Diabetic	1.04 ± 0.04	0.5 ± 0.01	0.54 ± 0.03	51.74 ± 1.38
48	Non diabetic	0.91 ± 0.04	0.45 ± 0.01	0.46 ± 0.03	50.92 ± 1.37
	Diabetic	0.75 ± 0.02	0.41 ± 0.02	0.34 ± 0.02	45.13 ± 1.30
72	Non diabetic	0.73 ± 0.02	0.39 ± 0.01	0.34 ± 0.03	46.36 ± 2.67
	Diabetic	0.51 ± 0.01	0.35 ± 0.01	0.16 ± 0.01	32.03 ± 1.97

Note: percentage of reduction was calculated using formula $C = 100 - (B/A * 100)$.

methyltransferase. In contrast, the adaptive response did not play such a large role in the repair of O6-ethylguanine and O4-ethylthymine, because ethylation of DNA is a poor inducer of the adaptive response. Contrary to this Mahmood and Vasudev [29] and Vasudev et al. [37] has demonstrated ethylating agents as efficient inducer of adaptive response than methylating agents in *P. pictus* i.e. insect system. Hence, further understanding of this phenomenon is needed using different alkylating agents and varied test systems.

It is worthy to note in the present investigations that there is an increased chromosomal break with combined treatment of both the tested agents in diabetic mouse compared to non diabetic mouse (Tables 1–5). Thus it is hypothesized that adaptive response is not efficiently induced by tested agents in diabetic mouse than that of non diabetic. This is the first report in the literature and hence further proof is required to decipher the same with some more experiments. However, it can be mentioned here that, Blasiak et al. [38] when treated lymphocytes of healthy and diabetic patients with hydrogen peroxide or doxorubicin and incubated with three different repair enzymes such as endonuclease III, formamidopyrimidine-DNA glycosylase and 3-methyladenine-DNA glycosylase II, showed decreased DNA repair in diabetic compared to control. Simone et al. [39] have also demonstrated the decreased expression of 8-oxo G-DNA glycosylase (OGG), a DNA repair enzyme, in kidney of diabetic rats and Akcay et al. [40] of O-6 methyl guanine methyltransferase (MGMT) activity in leukocytes of diabetic patients.

Studies have revealed that the adaptive response is manifested up to the third mitosis, and later due to dilution of the repair system as the cells divide into subsequent cell cycles, there is vanishing of adaptive response [41,42]. It is further strengthened by the observations of Mahmood et al. [25], Harish et al. [43], Guruprasad and Vasudev [44], Guruprasad et al. [36], where they have shown the reduction in frequency of chromosomal aberrations at 72 h RT, i.e. third subsequent mitosis in normal mouse bone marrow cells compared to 24 h and 48 h RTs. The present investigations are also on par with the earlier results in that the percentage reduction of chromosomal breaks with combined treatment of MMS is decreased from 51.74% at 24 h to 32.03% at 72 h RT in diabetic mouse, similar observations were made with EMS also (Tables 6 and 7). Similarly it can also be noted that the lowest breaks were seen at 72 h than at 24 and 48 h RTs, induced by conditioning and challenging doses of both the tested agents (Fig. 1). This agrees with earlier reports of Obe and Beek [45], where it has been amply proved that the decrease in aberration frequency with increasing culturing time reflects a mechanism of mitotic selection of aberration bearing cells.

In conclusion, it can be stated that (1) both EMS and MMS induced adaptive response not only in non diabetic mouse but also in diabetic mouse. (2) Further, decrease in chromosomal breaks in diabetic mouse compared to non diabetic indicated reduced reparability in diabetic mice. (3) And also ethylating and methylating agents showed differential adaptive response to chromosome damages in diabetic mice.

Conflict of interest

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

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