

## Full Length Research Paper

# Protective role of interferon against cytotoxicity induced by rabies virus in mice

Tohamy A. A.<sup>1</sup>, Fahmy A. M.<sup>2</sup>, Dkhil M. A.<sup>1,3\*</sup> and Diab M. S. M.<sup>1</sup>

<sup>1</sup>Zoology and Entomology Department, Faculty of Science, Helwan University, Egypt.

<sup>2</sup>Applied Research Sector, EGYVAC- VACSERA, Egypt.

<sup>3</sup>Zoology Department, College of Science, King Saud University, P.O. Box: 2455, Riyadh- 11451, Saudi Arabia.

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**Rabies remains an important public health problem in the world due to uncontrolled enzootic rabies, lack of safe efficient vaccines and poor information on the risk of contracting rabies post animal exposure. The lethality and mutagenic potential of challenge virus standard (CVS) was evaluated in mice. Mice were intracerebrally infected (MIC) with low, medium and high viral LD<sub>50</sub> (MICLD<sub>50</sub>). Mice were subjected to immunomodulation using interferon (IFN $\alpha$ -2a) pre- infection. The infected groups pretreated with IFN- $\alpha$  2a showed a higher survival rate than the infected group. Statistically significant increase in structural and numerical chromosomal aberrations and a decreased mitotic activity of mice bone marrow cells was observed post infection. Pretreatment of the infected groups with IFN  $\alpha$ -2a showed a marked and significant reduction of these cytogenetic changes. The increased survival rate and reduced cytogenetic changes suggest that protection induced by interferon against rabies virus activity could be, at least partially, attributable to blockage of the replication of CVS strain of rabies virus. It could be concluded that interferon can be used as an immune enhancer to the application of vaccine administration.**

**Key words:** Rabies, interferon, chromosomal aberration.

## INTRODUCTION

Rabies is a zoonotic disease caused by RNA virus from family Rhabdoviridae, genus Lyssavirus (Manning et al., 2008; Faber et al., 2009; Ogawa et al., 2009). Most human deaths from rabies occur in tropical resource

limited countries (Warrell and Warrell, 1991). In Africa and Asia, an estimated 24,000 to 70,000 people die of rabies each year (Knobel et al., 2005). The domestic dog is the main source of exposure and a primary vector for human rabies (Wandeler et al., 1993). The transmission of rabies virus occurs mainly by contact with the saliva of a rabid animal. Dogs, cats, foxes, bats, raccoons, skunks, coyotes, monkeys and wolves are among the most risky animals that can transmit rabies (Hendekli, 2005). The most common site of rabies virus (RV) entry in humans is the skin or mucous membrane, where the virus is delivered into the muscle and subcutaneous tissue through biting, licking or scratching by an RV-infected animal (Warrell, 2004). The fact that viruses can act as mutagens was first reported by Alikhanian and Iijina (1958) in their work on the induction of mutation in actinomycetes by phages. The exact mechanism of viral mutagenesis is not well known. Viruses may attack

\*Corresponding author. E-mail: mohameddkhil@yahoo.com.  
Tel: 00966-14675754 (office).

**Abbreviations:** RV, Rabies virus; IFN, interferon; CA, chromosomal aberrations; CVS, challenge virus standard; HrIFN- $\alpha$ 2a, Human Interferon alpha-2a; I/P, intraperitoneally; CP, cyclophosphamide; FCS, fetal calf serum; PCEs, polychromatic erythrocytes; NCEs, normochromatic erythrocytes; MN, micronuclei; DPI, day post infection; PCE%, polychromatic erythrocytes percentage; MNPCEs‰, micronucleated polychromatic erythrocytes per thousand; MNCEs ‰, micronuclei in normochromatic erythrocytes per thousand.

chromosomes directly or cause activation of lysosomal enzymes including DNase and cathepsin. Light induced selective activation of lysosomal enzymes produces increased chromosomal breakage, either directly or by destroying the proteins associated with the chromosomes (Allison and Panton, 1965). This mechanism has not yet been proved, but may be true for certain viral pathogens. Rabies virus was found to induce apoptotic changes in brain neurons (Jackson, 1999; Baloul and Lafon, 2003; Suja et al., 2009). It is known that mice, hamsters, or rabbits infected with rabies virus can be protected from the disease by administering interferon or inducing interferon by polyriboinosinic polyribocytidylic acid in the animals around the time of infection (Janis and Habel, 1972). The present study is an attempt to investigate the mutagenic potential of rabies virus infection in mice and the possible inhibitory effect of interferon (IFN) on this viral activity as well as its clastogenic effects.

## MATERIALS AND METHODS

### Animals

Adult male Swiss albino mice aged from 3 to 5 months and weighing 20 - 25 gm were purchased from Research Institute of Ophthalmology (Giza, Egypt), housed in specially designed cages and kept in the laboratory under the normal light-dark rhythm, food and water supplement for at least one week before starting the experiment. All experiments were performed in accordance with the Egyptian Animal Protection Law and were approved by the Animal Research Ethics.

### Rabies virus

The challenge virus standard (CVS) strain of fixed rabies virus was used in three different concentrations of the virus titer. The CVS strain was kindly supplied by Dr. Aly Fahmy Mohamed (Rab. Vac. Res. unit, VACSERA, Egypt). Mice were intracerebrally infected with viral lethal dose fifty (MICLD<sub>50</sub>) calculated according to the method of "Reed and Muench, 1938". Low, intermediate and high of CVS concentration contained 1 MICLD<sub>50</sub>, 10 MICLD<sub>50</sub>, 100 MICLD<sub>50</sub>, respectively.

### Interferon

Human Interferon alpha- 2a (HrIFN- $\alpha$  2a) ( Hoffmann-La Roche, USA) was subcutaneously injected in mice 24 h pre viral infection with the three different concentrations of the CVS in a single dose equal to 300 IU/0.5 ml of MEM-E medium.

### Animal groups

Mice were divided into 9 groups of 10 mice each. Group 1 served as vehicle control group in which mice were intracerebrally inoculated with medium free of Hr-IFN $\alpha$ -2a (0.03 ml /mouse). Group 2 were subcutaneously injected with 300 IU/0.5 ml of Hr-IFN $\alpha$ -2a contained in 0.5 ml of medium /mouse. Groups 3, 4 and 5 were inoculated 300 IU/0.5 ml of Hr-IFN $\alpha$ -2a in MEM-E medium 24 h pre-CVS intracerebral inoculation as low, medium and high concentration; 1, 10 and 100 MICLD<sub>50</sub>, respectively. Group 6, 7 and 8

were inoculated with the low, medium and high dose of CVS as positive control for the CVS-IFN treated groups. Group 9 were given a single intraperitoneally (I/P) injection of cyclophosphamide (CP) as a mutagenic agent obtained in form of water soluble commercial Endoxan (Asta, Germany), used as 40 mg/kg mouse body weight. CP was used as a mutagenic agent (Ramesh and Pramod, 2003).

### Chromosomal preparations

Bone marrow preparations for the analysis of chromosomal aberrations in metaphase cells were carried out on day 5 post viral infection according to Yosida and Amans (1965). In brief, mice were injected intraperitoneally with 0.04% colchicines purchased from Sigma-Aldrich (Buchs SG, Switzerland). After 120 min they were sacrificed by cervical dislocation. Bone marrow cells were collected in saline solution and then transferred to hypotonic solution (0.075 M KCL) for 20 min at 37°C and then fixed with cold fixative (3 part methanol to 1 part glacial acetic acid). Chromosomes were stained with Giemsa. Fifty metaphase cells were scored per mouse to determine the mean numbers of total chromosomal aberrations.

### Mitotic index

For each mouse, 1000 cells were counted and the number of dividing cells including late prophase and metaphases were determined, the mitotic index was computed as the ratio of the dividing cells to the total examined cells multiplied by 100.

### Micronucleus assay

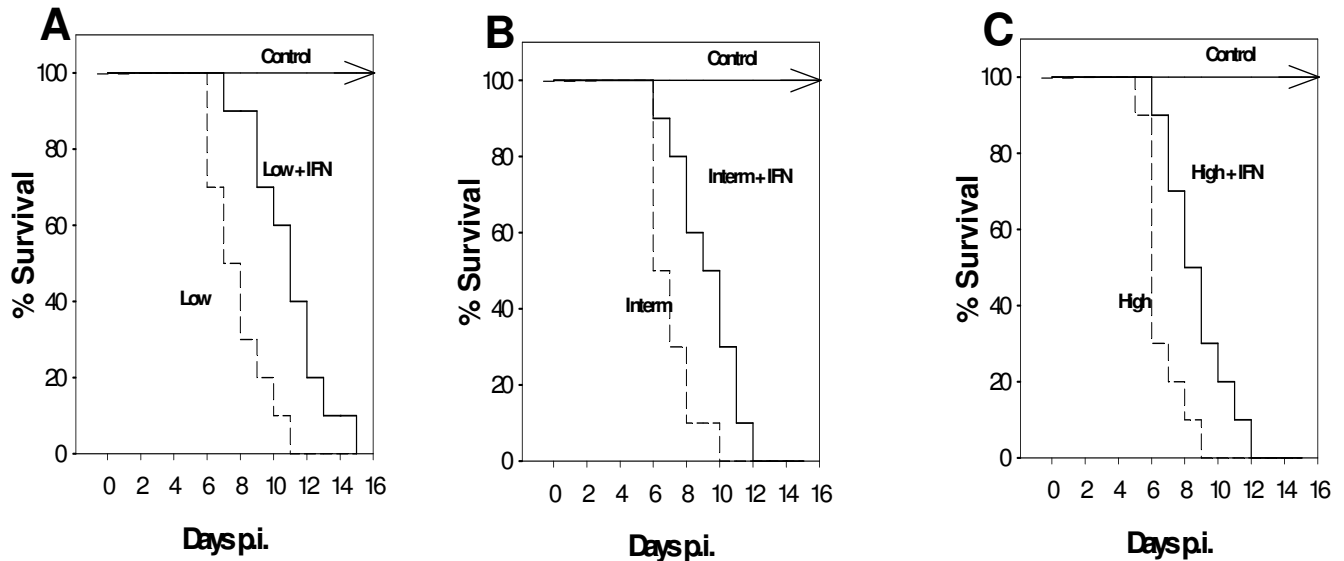
Bone marrow smears were prepared according to the method of Salamone and Heddle (1983) with slight modifications. In brief, the proximal ends of the femur were carefully cut and the marrow was pushed directly onto the slide and mixed with a drop of fetal calf serum (FCS) found previously on the same slide and then the cells dispersed with the edge of a second clean slide which is subsequently used to spread the smear. Slides were normally air-dried and fixed for 2 - 5 min in absolute methanol. Slides were stained with May-Gruenwald/Giemsa solution for 20 min and then mounted with DPX. The relative proportions of the polychromatic erythrocytes (PCEs) and the normochromatic erythrocytes (NCEs) observed at each 100 erythrocytes was counted until the total reached 1000. The micronuclei (MN) were recognized as deep purple stained bodies in the cytoplasm.

### Statistical analysis

Statistical analysis of cytogenetic data was carried out by using one way analysis of variance (ANOVA) followed by Post hoc test (LSD) using the Statistical Package for the Social Sciences (SPSS) version 10. Histograms of cytogenetic data were drawn using Excel 2003 (Microsoft, USA). Survival rate curves were drawn using Sigma Plot 2001 (SPSS, USA).

## RESULTS

Post infection with the three concentrations of CVS showed some characteristic morphological changes in mice within 14 day in the form of ruffling of hair, tremors, in coordination, paralysis of hind and fore limbs and finally death. These symptoms were more pronounced on



**Figure 1.** Survival percentage after different treatments. A: % Survival post infection with low CVS concentration (1 MICLD<sub>50</sub>) alone or combined with IFN $\alpha$ -2a. B: % Survival post infection with intermediate CVS concentration (10 MICLD<sub>50</sub>) alone or combined with IFN $\alpha$ -2a. C: % Survival post infection with high CVS concentration (100 MICLD<sub>50</sub>) alone or combined with IFN $\alpha$ -2a.

8, 7 and 6 day post infection (DPI) for low, intermediate and high concentrations of CVS, respectively.

Treatment of mice with single subcutaneously injection of IFN $\alpha$ -2a, 24 h before infection with the three concentrations of CVS delayed the onset of specific rabies signs but could not prevent them.

Figures 1a, 1b and 1c represented the percentage of mice survival post CVS infection with the three concentrations either pretreated with IFN $\alpha$ -2a or not, 100% mortality in non IFN treated infected mice was recorded on 11<sup>th</sup>, 10<sup>th</sup> and 9<sup>th</sup> DPI corresponding to low, intermediate and high concentrations of CVS, respectively. While mortality rates of infected mice pretreated with IFN $\alpha$ -2a were 90, 100 and 100% on 14<sup>th</sup>, 13<sup>th</sup> and 12<sup>th</sup> DPI for low, intermediate and high concentrations of CVS, respectively.

### Induction of chromosomal aberrations

Structural chromosomal aberrations observed in the present study were in the form of chromatid breakage, deletions, breaks, fragments, gaps, rings, centric fusions, end to end associations and centromeric attenuations. A cell was considered centromerically attenuated when it contains at least three chromosomes with centromeric split. While numerical chromosomal aberrations observed were in the form of endomitosis and polyploidy (Figure 2).

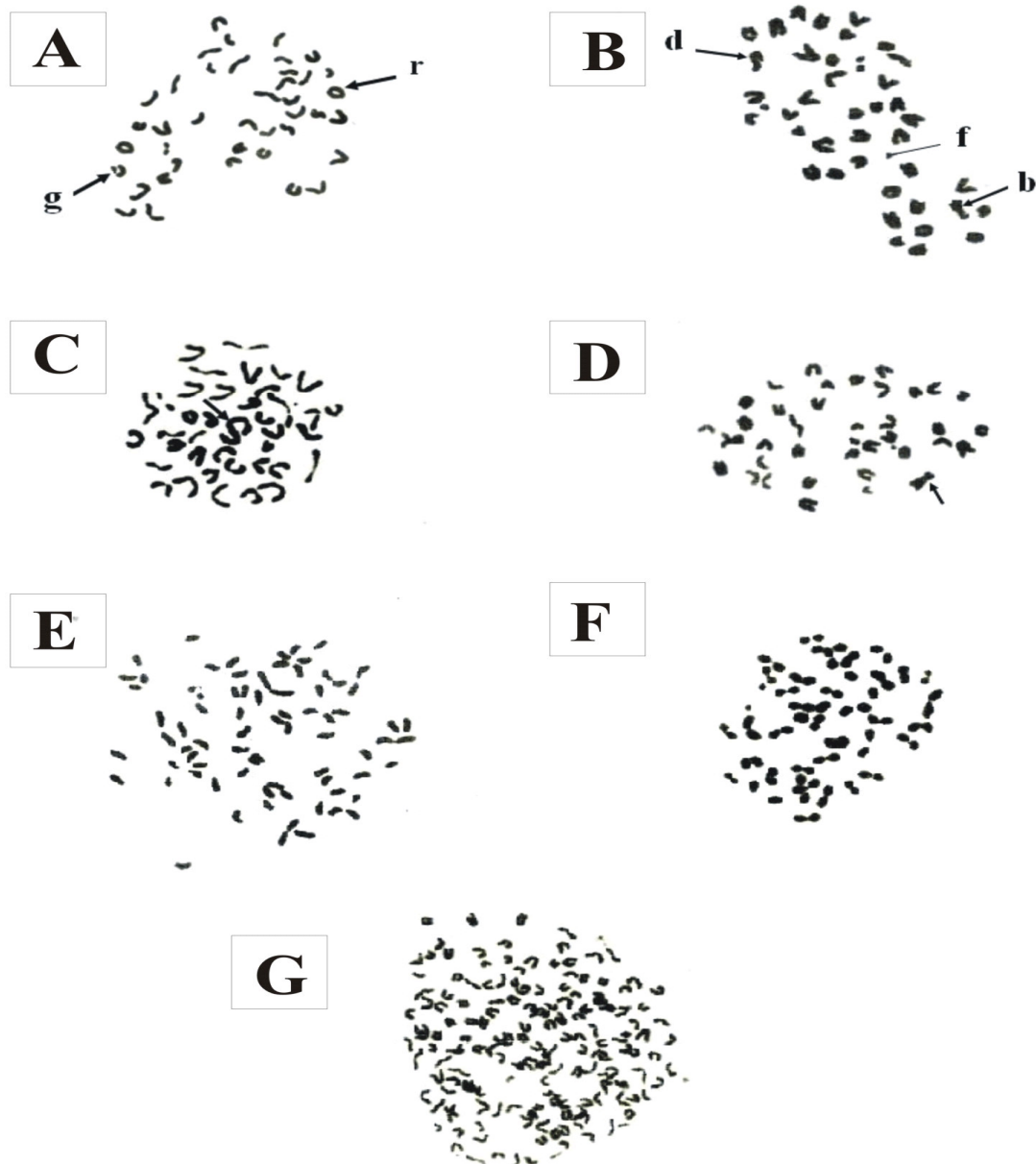
The data obtained in the present study revealed that subcutaneous administration of IFN $\alpha$ -2a alone to normal mice induced a non significant increase in the mean numbers of all observed types of structural and numerical chromosomal aberrations versus that of vehicle control group.

Conversely, the results of positive control group intraperitoneally injected with CP induced a sharp and marked significant elevation in mean numbers of all observed types of structural CAs ( $P < 0.001$ ) and a non significant increase in mean numbers of numerical CAs compared to the vehicle control group (Table 1).

A highly significant increase post infection with low concentration of CVS was recorded in the mean numbers of chromatid breaks, centric fusions, end to end associations and total structural CAs ( $P < 0.001$ ) and in that of rings ( $P < 0.01$ ) versus the vehicle control group while, there were a non-significant increase in the mean number of deletions, fragments, gaps, centromeric attenuations, endomitosis, polyploidy and total numerical chromosomal aberrations versus the vehicle control group (Figure 3).

In addition, administration of IFN $\alpha$ -2a 24 h before viral infection induced a marked and significant decrease in the mean numbers of rings and total structural CAs ( $P < 0.01$ ) and in that of end to end associations ( $P < 0.001$ ), which showed a non-significant decrease in other types of structural CAs when compared to the infected group with low concentration of CVS alone. Moreover, IFN $\alpha$ -2a and low CVS concentration treated group recorded a highly and significant increase ( $p < 0.01$ ) in the mean numbers of total structural CAs versus that of treated group with IFN $\alpha$ -2a alone and the vehicle control group.

There was a highly significant increase post infection with intermediate concentration of CVS in the mean numbers of chromatid breaks, centric fusions, rings, end to end associations, fragments, total structural and total numerical (endomitosis) CAs ( $P < 0.001$ ) and in that of gaps, centromeric attenuations and deletions ( $P < 0.01$ ) versus vehicle control group. On the other hand, admini-



**Figure 2.** A photomicrograph of metaphase spreading from mouse bone marrow showing A = ring chromosome (r) and gap (g); B = chromatid break (b), terminal deletion (d) and fragmented chromosome (f); C = end to end association (arrow); D = centric fusion (arrow); E = centromeric attenuation; F = endomitosis; G = polyploidy.

stration of IFN $\alpha$ -2a 24 h pre infection with intermediate concentration of CVS induced a marked and significant decrease in the mean numbers of chromatid breaks, centric fusions, rings, and total structural CAs ( $P < 0.01$ ) and in that of end to end associations, fragments, and total numerical (endomitosis) CAs ( $P < 0.001$ ) when compared to low concentration of CVS alone infected group. Moreover, pretreatment with IFN $\alpha$ -2a, the low concentration of CVS infected group induced a highly and significant increase in the mean numbers of total structural CAs ( $P < 0.01$ ) and ( $P < 0.001$ ) versus that of IFN $\alpha$ -2a group

and the vehicle control group, respectively (Table 1 and Figure 3).

Infection of mice with a high concentration of CVS showed a highly significant increase in the mean numbers of gaps, centromeric attenuations and deletions ( $P < 0.01$ ), chromatid breaks, centric fusions, rings, end to end associations, fragments, total structural and total numerical (endomitosis) CAs ( $P < 0.001$ ) post infection with high concentration of CVS versus the vehicle control group (Table 1 and Figure 2). On the opposite site, administration of IFN $\alpha$ -2a 24 h pre infection with high

**Table 1.** Chromosomal aberrations induced in mice bone marrow cells post infected with rabies virus.

Types of chromosomal aberrations	Dose	Vehicle control	IFN $\alpha$ -2a	MICLD <sub>50</sub>	IFN $\alpha$ -2a + MICLD <sub>50</sub>	Positive control (CP)
Chromatid gaps	L	0.2±0.447	0.2±0.447	0.6±0.548	0.4±0.548	1.2±0.83 <sup>a*</sup>
	M	0.2±0.447	0.2±0.447	1±0.707 <sup>a*</sup>	0.4±0.548	1.2±0.83 <sup>a*</sup>
	H	0.2±0.447	0.2±0.447	1±0.707 <sup>a*</sup>	0.4±0.548	1.2±0.83 <sup>a*</sup>
Chromatid break	L	0.2±0.447	0.4±0.548	1.8±0.837 <sup>a**</sup>	1.2±0.447	1.6±1.140 <sup>a**</sup>
	M	0.2±0.447	0.4±0.548	2.2±0.448 <sup>a**</sup>	1±0.707 <sup>c*</sup>	1.6±1.140 <sup>a**</sup>
	H	0.2±0.447	0.4±0.548	3±1.225 <sup>a**</sup>	2.2±1.304 <sup>a**b**</sup>	1.6±1.140 <sup>a**</sup>
Centromeric fusions	L	0.2±0.447	0.4±0.548	1.2±0.837 <sup>a**</sup>	0.6±0.548	5.4±0.894 <sup>a**</sup>
	M	0.2±0.447	0.4±0.548	1.6±0.894 <sup>a**</sup>	0.6±0.548 <sup>c*</sup>	5.4±0.894 <sup>a**</sup>
	H	0.2±0.447	0.4±0.548	3.6±1.140 <sup>a**</sup>	1±0.707 <sup>c**</sup>	5.4±0.894 <sup>a**</sup>
Centromeric attenuation	L	0.4±0.548	0.8±0.837	1±0.707	0.8±0.447	1±0.707
	M	0.4±0.548	0.8±0.837	1.4±0.548 <sup>a*</sup>	0.6±0.548	1±0.707
	H	0.4±0.548	0.8±0.837	1.4±0.548 <sup>a*</sup>	1.2±0.447	1±0.707
Ring chromosome	L	0	0.2±0.447	1.4±0.548 <sup>a*</sup>	0.2±0.447 <sup>c*</sup>	3.8±0.837 <sup>a**</sup>
	M	0	0.2±0.447	1.6±0.548 <sup>a**</sup>	0.6±0.548 <sup>c*</sup>	3.8±0.837 <sup>a**</sup>
	H	0	0.2±0.447	1.8±0.837 <sup>a**</sup>	1±0.707 <sup>a*b*c*</sup>	3.8±0.837 <sup>a**</sup>
End to end association	L	0	0	1.2±0.447 <sup>a**</sup>	0.2±0.447 <sup>c**</sup>	0.6±0.548 <sup>a*</sup>
	M	0	0	1.6±0.548 <sup>a**</sup>	0.4±0.548 <sup>c**</sup>	0.6±0.548 <sup>a*</sup>
	H	0	0	2±0.707 <sup>a**</sup>	1.4±0.548 <sup>a**b*c*</sup>	0.6±0.548 <sup>a*</sup>
Deletions	L	0	0	0.2±0.447	0.2±0.447	0.6±0.548 <sup>a*</sup>
	M	0	0	0.8±0.448 <sup>a*</sup>	0.2±0.447	0.6±0.548 <sup>a*</sup>
	H	0	0	0.8±0.837 <sup>a*</sup>	1±0.707 <sup>a*b*</sup>	0.6±0.548 <sup>a*</sup>
Fragments	L	0	0	0.4±0.548	0	1.6±0.548 <sup>a**</sup>
	M	0	0	1.6±0.894 <sup>a**</sup>	0 <sup>c**</sup>	1.6±0.548 <sup>a**</sup>
	H	0	0	1.6±1.140 <sup>a**</sup>	0.6±0.548 <sup>c*</sup>	1.6±0.548 <sup>a**</sup>
Endomitosis	L	0	0.4±0.548	0.4±0.548	0.4±0.548	0.8±0.837
	M	0	0.4±0.548	1.4±1.140 <sup>a**</sup>	0.4±0.548 <sup>c**</sup>	0.8±0.837
	H	0	0.4±0.548	1.8±0.837 <sup>a**</sup>	1±0.707 <sup>a*</sup>	0.8±0.837
Polyploidy	L	0	0	0	0	0.2±0.447
	M	0	0	0.4±0.548	0.2±0.447	0.2±0.447
	H	0	0	0.4±0.548	0.4±0.548	0.2±0.447
Total numerical aberrations	L	0	0.4±0.548	0.4±0.548	0.4±0.548	1±1.225
	M	0	0.4±0.548	1.8±1.304 <sup>a**</sup>	0.6±0.894 <sup>c**</sup>	1±1.225
	H	0	0.4±0.548	2.2±1.096 <sup>a**</sup>	1.4±0.548 <sup>a**b*</sup>	1±1.225

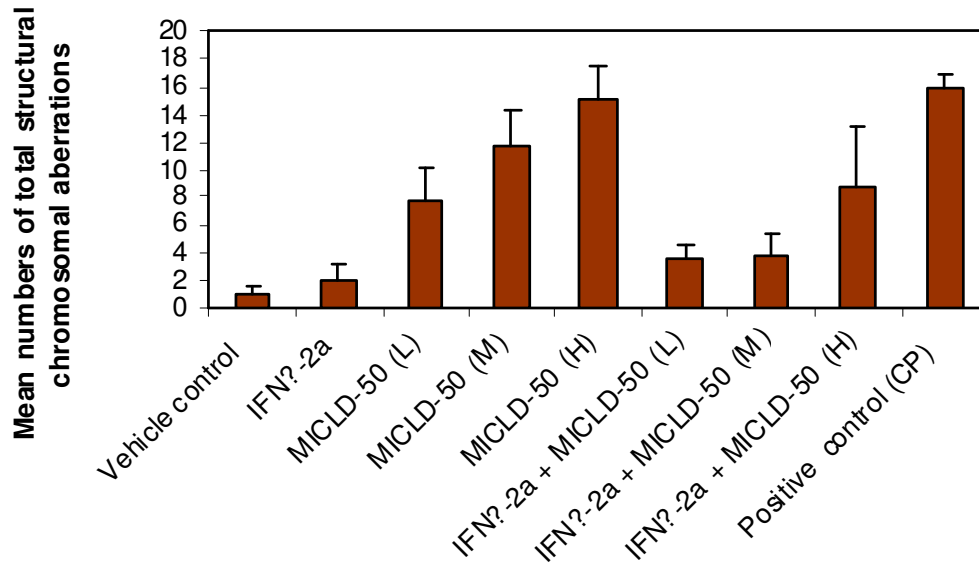
Data were expressed as Mean  $\pm$  Standard Deviation (M $\pm$ SD) values of number of each aberration/250 metaphase/group; number of animals = 5 mice/each group; number of examined metaphases = 50 cell / mouse.

CP = cyclophosphamide; a = significant change at P < 0.05 with respect to vehicle control group; b = significant change at P < 0.05 with respect to IFN $\alpha$ -2a group; c = significant change at P < 0.05 with respect to CVS concentration infected group; \* = statistically significant at P < 0.01; \*\* = statistically significant at P < 0.001.

MICLD<sub>50</sub> = mice intracerebrally infected with viral lethal dose fifty; L = low dose (1 MICLD<sub>50</sub>); M = intermediate dose (10 MICLD<sub>50</sub>); H = high dose (100 MICLD<sub>50</sub>).

concentration of CVS induced a marked and significant decrease in the mean numbers of induced rings, end to end associations and fragments (P < 0.01) and in that of centric fusion and total structural CAs (P < 0.001) while a non-significant decrease in total numerical CAs (P > 0.05) was clearly observed when compared with high concentration of CVS infected group. Moreover,

combined treatment of IFN $\alpha$ -2a with high concentration of CVS infected group induced a highly and significant increase (P < 0.01) in the mean numbers of break, rings, end to end associations, deletions, total structural CAs and total numerical CAs (endomitosis) versus that of IFN $\alpha$ -2a group and the vehicle control group (Table 1 and Figure 3).



**Figure 3.** Total structural chromosomal aberrations induced in mice bone marrow cells post infected with rabies virus alone or combined with IFN $\alpha$ -2a. Low CVS concentration = 1 MICLD50; intermediate CVS concentration = 10 MICLD50; high CVS concentration = 100 MICLD50.

### Changes induced in mitotic index due to rabies infection

A non significant decrease ( $P > 0.05$ ) in percentage of MI (2.40%) was recorded in normal mice S/C inoculated with IFN $\alpha$ -2a versus that of the vehicle control group (2.60%). On the other hand, a very highly significant decrease ( $P < 0.001$ ) was observed in percentage of MI (1.68, 1.60 and 1.48%) post infection with (low, intermediate and high) concentrations of CVS, respectively, when compared with that of the vehicle control group. Administration of IFN $\alpha$ -2a pre infection with low, intermediate and high concentrations of CVS induce a marked and significant increase ( $P < 0.001$ ) in percentage of MI 2.20, 2.21 and 1.86%, respectively, versus that of same CVS concentrations infected groups alone. A marked significant decrease ( $P < 0.001$ ) in percentage of MI was recorded in all groups of IFN $\alpha$ -2a combined with CVS infection as compared to that of the vehicle control group (Figure 4).

### Changes in micronucleated bone marrow cells due to rabies infection

Mice intrapretoneally injected with CP induced a sharp and marked significant elevation of both polychromatic erythrocytes percentage (PCE %), micronucleated polychromatic erythrocytes per thousand PCEs (MNPCEs ‰) and micronuclei in normochromatic erythrocytes per thousand NCEs (MNNCEs ‰) at  $P < 0.001$  with a percentage of 74.41 10.40 and 1.16‰, respectively, as compared to that of the vehicle control group (Table 2).

Inoculation of low, intermediate and high CVS concen-

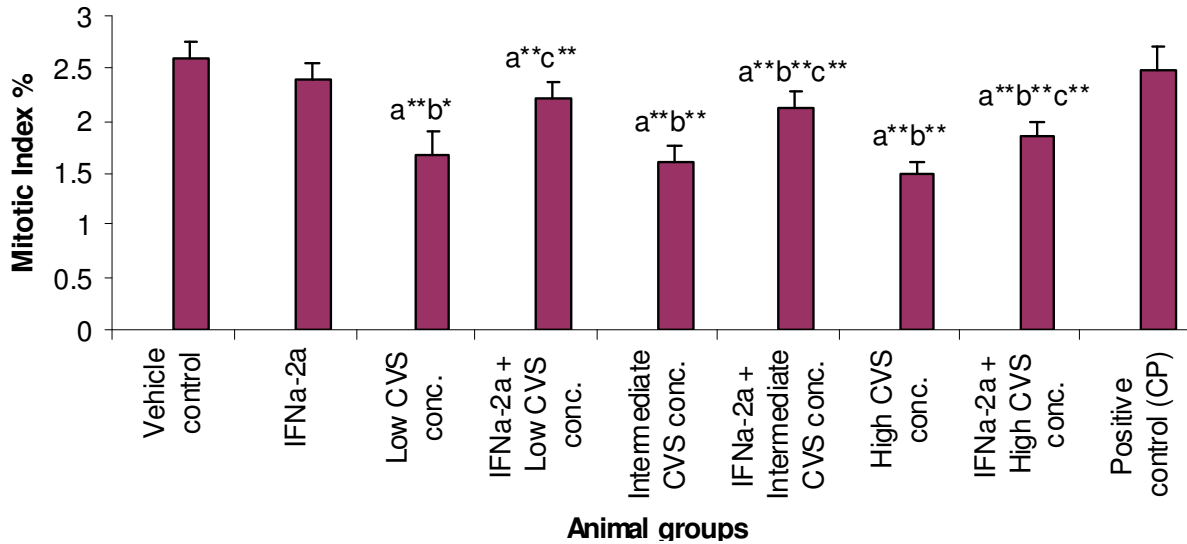
trations induced a highly significant increase of both PCEs% and MNPCEs ‰ ( $P < 0.001$ ). Also, the incidence of MNNCEs ‰ was significantly elevated ( $P < 0.01$ ) as compared to the vehicle control group (Table 2). Pretreatment of mice with IFN $\alpha$ -2a before infection with CVS concentrations showed a significant reduction in PCEs% and MNPCEs ‰ when compared to the infected groups (Table 2).

### DISCUSSION

Some viruses cause random aberrations whilst others induce specific alterations to one or more of the chromosomes (Steffensen et al., 1976; Matsuoka and Jeang 2007; Lee et al., 2009). Rabies virus was initially reported to induce random damage in mice bone marrow cells (Stich and Yohn, 1970).

In the present study, infection of mice intracerebrally with three different concentration of CVS strain of rabies virus led to high mortality rates between infected mice where mice infected with low, intermediate and high CVS concentrations showed 100% mortality at the 11<sup>th</sup>, 10<sup>th</sup> and 9<sup>th</sup> DPI, respectively. These results were in agreement with that recorded in the previous studies by Marcovistz et al. (1984) who reported that rabies virus infection in mice inoculated with CVS strain (1LD<sub>50</sub>) was characterized by paralysis of the posterior limbs on day 5 post infection and this infection was lethal to all mice by day 7 or 8 post infection.

In the present study, cyclophosphamide induced a very high percentage of aberrant metaphases and highly significant number of structural chromosomal aberrations ( $p \leq$



**Figure 4.** Mitotic activity of mice bone marrow cells of mice infected with rabies virus alone or combined with IFN $\alpha$ -2a. Low CVS concentration = 1 MICLD<sub>50</sub>; intermediate CVS concentration = 10 MICLD<sub>50</sub>; high CVS concentration = 100 MICLD<sub>50</sub>; a = significant change at  $p < 0.05$  with respect to vehicle control group; b = significant change at  $p < 0.05$  with respect to IFN $\alpha$ -2a group; c = significant change at  $p < 0.05$  with respect to same CVS concentration alone; \*, \*\* = changes at  $p < 0.01$  and  $p < 0.001$ , respectively.

**Table 2.** Frequencies of micronucleated bone marrow cells of mice post infected with rabies virus.

Animal groups		Number of examined PCEs	% PCEs	% MNPCEs	% MNNCEs
Vehicle control		5000	52.75	0.60	0.00
IFN $\alpha$ -2a		5000	52.85	1.00	0.00
MICLD <sub>50</sub>	L	5000	62.46 <sup>a**</sup>	5.40 <sup>a**</sup>	0.34
	M	5000	64.58 <sup>a**</sup>	8.00 <sup>a**</sup>	1.09 <sup>a*</sup>
	H	5000	70.84 <sup>a**</sup>	11.00 <sup>a**</sup>	1.94 <sup>a*</sup>
IFN $\alpha$ -2a + MICLD <sub>50</sub>	L	5000	57.71 <sup>a**b**c**</sup>	1.60 <sup>c**</sup>	0.27
	M	5000	59.10 <sup>a**b**c**</sup>	3.20 <sup>a**b**c**</sup>	0.58
	H	5000	59.60 <sup>a**b**c**</sup>	7.20 <sup>a**b**c*</sup>	0.89
Positive control (CP)		5000	74.41 <sup>a**</sup>	10.40 <sup>a**</sup>	1.16 <sup>a*</sup>

a = Significant change at  $p < 0.05$  with respect to vehicle control group; b = significant change at  $p < 0.05$  with respect to IFN $\alpha$ -2a group; c = significant change at  $p < 0.05$  with respect to same CVS concentration group; \* = statistically significant at  $P < 0.01$ ; \*\* = statistically significant at  $P < 0.001$ .

0.001) in male mice. This recorded data was in complete agreement with earlier reports (Datta and Schliermacher, 1969; Rohrborn and Basler, 1977; Machemer and Lorke, 1978; Iarc, 1981; Valadares et al., 2007; Berekati et al., 2008; Tripathi and Jena, 2009).

Also, in the present study, the infection of mice with the three different concentrations of rabies virus CVS showed a significant increase in both structural and numerical chromosomal aberrations and in micronuclei as well, while the mitotic activity of mice bone marrow cells were decreased. Rabies virus had a clastogenic effect (Cherkezia et al., 1980; Nayak and Das, 1987; Rao and Polesa, 1991). Where there was a significant increase in

structural chromosomal aberrations, it was mainly due to significant increase in centromeric fusion, breaks, rings and centromeric attenuation (except after low concentration). Centromeric attenuation may lead to a concomitant increase in endomitosis and polyploidy (de Hondt et al., 1984).

The observed highly significant increase in micronuclei formation in both PCEs and NCEs may be attributed to significant increase in breaks in which micronuclei formation is a consequence of chromosomes and spindle apparatus damage in cells.

The significant increase in numerical chromosomal aberrations was mainly due to a significant increase in

endomitosis after intermediate and high concentrations of CVS.

The significant decrease in the mitotic activity of mice bone marrow cells post viral infection indicates that the rabies virus interacted with the spindle apparatus and this result is in agreement with the fact that viruses are known to cause spindle disturbance similar to those caused by colchicine (Stich, 1969; Ceccaldi et al., 1990).

The exact mechanism of viral mutagenesis is not known. Viruses may attack chromosomes directly. Certain viruses cause activation of lysosomal enzymes including DNase and cathepsin. Light induced selective activation of lysosomal enzymes produces increased chromosomal breakage, either directly or by destroying the proteins associated with the chromosomes (Allison and Panton 1965; Li et al., 2005). This mechanism has not yet been proved, but may be true for certain viral pathogens. Further studies are essential to unveil the exact mechanism of the clastogenic action of different viruses on the hereditary materials of the inoculated organisms.

Pretreatment of mice with IFN $\alpha$ -2a 24 h pre infection with rabies virus can delay the mortality of mice and induced a significant cytogenetic changes post infection with rabies virus after pretreatment with IFN $\alpha$ -2a. Interferon can inhibit cell growth and thereby inhibit the replication of some viruses by inducing Mx proteins which are highly conserved, large GTPases with homology to dynamin and have been found in all vertebrate species examined so far, including mammals (Staeheli et al., 1993; Arnheiter et al., 1995). Mx proteins interfere with virus replication, probably by inhibiting the trafficking or activity of virus polymerases (Stranden et al., 1993), thereby impairing the growth of a wide range of RNA viruses at the level of virus transcription and at other steps in the virus life-cycle.

In conclusion, results discussed here summarized the positive role of INF in delaying the incidence of mortality and in addition, the reduction of severe clastogenic damages. Further investigations are substantial for the evaluation of the role of interferon in case infection with rabies virus as an enhancer to vaccine delayed administration to minimize the incidence of mortality. In addition, evaluation of multi dose regimen, concentration and administration intervals of IFN should be carried out.

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