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Original Research Article

Imipramine Genotoxicity Research and the Effect of Vitamin E in Mice

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Abstract

Abstract: Evaluation of impiramine's genotoxicity and the function of vitamin E in mice is the goal. Materials & Procedures Seven groups of forty-two male albino mice were created at random (n=7). Group I served distiller water as the control group. Mice were given imipramine treatment in Groups (II-IV). Specifically, orally for two days in two split doses of 12.9 (¼LD_{50}), 25.8 (½LD_{50}) and 38.6 (¾ LD_{50}) mg/kg body weight. Groups (V, VI, and VII) of mice were administered with 100 mg/kg body weight of vitamin E orally every day for six days in order to assess the involvement of vitamin E in genotoxicity. The mice were subsequently given imipramine orally for five days. Animals in all the groups were sacrificed by euthanasia and were dissected and epididymal sperm sample was taken, smear was made and staining done, and screened under the microscope.

Results: A chi-square test was performed after the results. Using an assay for sperm head abnormalities, the current study. The protective effect of vitamin E was observed by a decrease in the incidence of abnormal sperms only in the Group V but not in Group VI or VII. Genotoxic effect of imipramine with ¼, ½, ¾LD_{50} doses were observed by an increase in incidence of abnormal sperms with the increasing dose of imipramine in the Groups II, III, & IV, compared to group I.

Conclusion: According to the sperm head abnormalities assay, imipramine had a genotoxic effect at all three of the levels mentioned—namely, ¼, ½, and ¾LD_{50}. Only for lower doses of imipramine does vitamin E have a geno protective effect.

Keywords: Genotoxicity, Imipramine & Vitamin E Imipramine and vitamin E.

Introduction

One of the most prevalent CNS disorders in the world is depression. In a population, the disease was prevalent at a rate of 14%. At best, barely 50% of depressed patients receive treatment, and among those who do, less than 50% of them experience recovery with available medications[1,2]. Depression can strike anyone at any age, from young children to the elderly. Across the entire world, tricyclic antidepressants (TCAs) are

the medications that are most frequently prescribed[3]. The likelihood of relapse is decreased in patients who continue their therapy, even though in certain situations the efficacy of TCAs in the acute phase of depression was lower than previously thought[4]. Infants whose mothers took the medication during the first trimester of pregnancy were found to have limb abnormalities[5-7]. In a different study, Barson[8] described a mother on long-term maintenance doses of imipramine who gave birth to a stillborn child with several rib abnormalities. It is a well-known fact that a rise in mutations could have the biggest impact on the creation of a genetic catastrophe in the next generation. Genetic damage can speed up the ageing process, induce cancer, and result in a wide range of hereditary diseases and pathological conditions. Vitamins E, A, C, and carotenoids are examples of antioxidants that are known to be reducing agents, which are chemicals that can stop other molecules from oxidising.

By eliminating free radicals, they stop the oxidative chain processes. Thus, the anti-genotoxic capability of these antioxidants has been amply proven in numerous prior research reports[8]. The current job has been completed in light of the importance described above. i.e., to examine imipramine's genotoxicity and the impact of vitamin E in mice.

Materials & Procedures

Materials

Animals: The animal house, Department of Pharmacology, provided 42 male Swiss albino mice weighing 25 to 30 gram's and 8 weeks old. As per the recommendations of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), all the animals were kept in standard conditions with appropriate food and water available at all times in the animal house. Before the experimental trial, the Institutional Animal Ethics Committee (IAEC) of Chalmeda Anand Rao Institute of Medical Sciences had approved the study (CAIMS/IAEC/2009).

Drugs:

Imipramine was sold by Torrent Laboratories as a 75 mg tablet. This dosage was based on the LD_{50} of imipramine in mice.

Vitamin E: MERK LTD's paediatric vitamin E drops, with a strength of 50 mg/ml, were used. And the dosage is 100 mg/kg body weight, which is administered orally and given for 6 days after imipramine.

Miscellaneous:

Microscope: For the screening of the slides under oil immersion.

Glass slides: Blood collected from the bone marrow were smeared on the glass slides for haematological study and also sperm sample from the epididymis were smeared on the glass slides for the study of morphology of sperms.

Pipette: Used for mixing the contents of sperm sample.

Petridish: Used to keep the dissected femur of the mice in the petridish containing normal saline and also epididymis which is dissected out from the mice also kept in a petridish containing saline.

Gauze: With the help of the gauze muscles present on the femur bone removed.

Dissecting instruments: Scissors and forceps are used to remove the femur from the mice.

The epididymis is sliced using a bent pair of scissors. % Eosin is used to stain the sperm sample taken from the epididymis.

The mouse epididymis and femur are kept in a petridish using 0.9% saline.

Methods

Abnormity in Sperm-Head[10]: Forty two male albino mice were chosen from the institution's animal house. The day of the experiment after an overnight fast with water at will. Seven groups of six mice each were created out of the mice.

Group-I: Control - received 0.5 ml of distilled water orally

Group-II: Imipramine 12.9mg/Kg body wt(¼ LD_{50})

Group-III: Imipramine 25.8mg/kg body wt(½LD_{50})

Group-IV: Imipramine 38.6mg/kg body wt(¾LD_{50})

Group-V: Vitamin E100mg/kg body wt + Imipramine 12.9mg/kg body wt

Group-VI: Vitamin E100mg/kg body wt + Imipramine 25.8mg/kg body wt

Group-VII: Vitamin E100mg/kg body wt + Imipramine 38.6mg/kg body wt

Groups II, III, and IV's animals were all sacrificed by cervical dislocation 35 days after the initial dose of imipramine was administered orally for five days in five different divided doses; however, in Groups V, VI, and VII, the animals first received Vitamin E 100 mg/kg body weight per day orally for six days before receiving imipramine for five days in five divided doses, which was then administered orally for five days after that. According to the procedure advised by Bruce *et al*. (1974), the mice from the control and various treatment groups were dissected, and epididymal sperm samples were tested on the 35th day.

Method for obtaining an epididymal sperm sample: Euthanasia (cervical dislocation) was used to kill all the animals, and the epididymis was then removed and placed in a petridish containing 0.9% normal saline. The epididymis was then minced for a few minutes, sliced with a pair of bent scissors, and its contents were then released with the aid of forceps. The suspension of the sperm sample was carefully mixed and filtered by aspiration and flushing with the Pasteur pipette. Following 1% Eosin staining of the filtered sperm suspension, smears were created on clean slides and mounted by DPX. To determine the impact of these medications on mouse germ cells, the mice from the control and various treatment groups were dissected, and epididymal sperm samples were tested on the 35th day in accordance with the approach advised by Bruce *et al* (1974).

Scoring

With an oil immersion microscope at a 100X magnification, the prepared slides were scanned. For each animal in the dosage group and control, 1000 sperms were counted.

Statistics

The mean and standard deviation were used to express the results. The chi-square test was used for statistical analysis. Statistics were deemed significant at $P< 0.05$.

Abnormal Sperm Head Test (Per 1000 Sperms)

Imipramine12.9mg/ kgbody wt (¼ LD_{50})

Imipramine25.8mg/ kg body wt (½ LD_{50})

Imipramine 38.6mg/ kg body wt (¾ LD_{50})

Vitamin E 100 mg/ kg/ body wt + Imipramine 12.9mg/kg body wt

Vitamin E 100 mg/ kg/ body wt + Imipramine 25.8mg/ kg body wt

Vitamin E 100 mg/ kg/ body wt + Imipramine38.6mg/kg body wt

Table 1: Abnormal Sperm Head Test (PER 1000 SPERMS)

S. No.	Control	Imipramine12.9mg/ kgbody wt (¼ LD50)	Imipramine25.8mg/ kgbody wt (½ LD50)	Imipramine 38.6mg/ kg body wt (¾LD50)	Vitamin E 100 mg/ kg/ body wt.+ Imipramine 12.9mg/kg body wt	Vitamin E 100 mg/ kg/ body wt.+ Imipramine 25.8mg/ kg body wt	Vitamin E 100 mg/ kg/ body wt.+ Imipramine38.6mg/k gbody wt
1	18	35	48	60	20	35	55
2	22	42	50	72	18	32	56
3	15	40	49	65	23	38	51
4	23	38	45	68	21	30	54
5	16	45	51	71	19	36	52
6	22	37	46	70	22	33	58
Mean	**19.33**	**39.5**	**48.16**	**67.6**	**20.5**	**34**	**54.33**
SD	**3.44**	**3.61**	**2.31**	**4.50**	**1.87**	**2.89**	**2.58**
SE	**1.40**	**1.47**	**0.946**	**1.844**	**0.766**	**1.184**	**1.057**

Table 2: Sperm Head Abnormality Test

Control, Imipramine 12.9mg/kg & VIT E (100 mg/kg + 12.9mg/kg)

Group(n=6)	Normal Sperms/1000	Abnormal Sperms /1000	% Of Abnormal Sperms
	Mean		
Control	980.7	19.33	1.93%
Imipramine 12.9mg/kg body wt (¼ LD50)	960.5	39.5**	3.95%
Vitamin E 100 mg/kg+ Imipramine 12.9mg/kg		20.5**	
body wt	979.5		2.05%

Mean±SD. *P*<0.001** compared to control SD: Standard deviation, Imipramine

Table 3: Sperm Head Abnormality Test

(Control, Imipramine 12.9mg/kg body wt, Vitamin E + Imipramine 12.9mg/kg body wt)

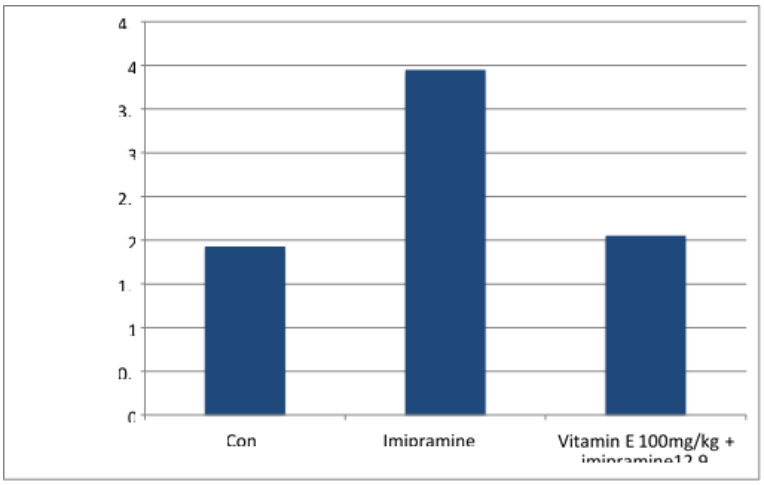
Group (n=6)	Normal Sperms/1000	Abnormal Sperms/1000	% Of Abnormal Sperms
	Mean		
Control	980.7	19.33	1.93%
Imipramine 12.9 mg/kgbody wt (½ LD50)	951.8	48.16***	4.81%
Vitamin E 100 mg/kg+ Imipramine12.9 mg/kg body wt	966	34	3.4%

P<0.05* P<0.01** P<0.001*** as compared to control

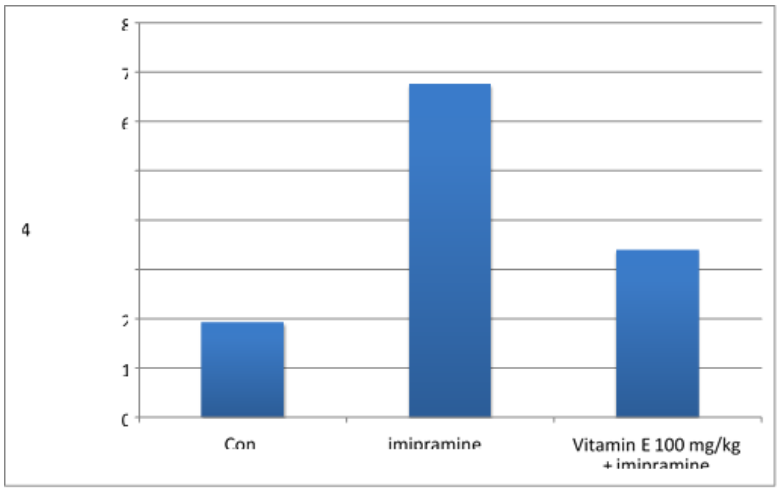
Table 4: Sperm Head Abnormality Test
(Control, Imipramine 38.6mg/kg body wt, Vitamin E + Imipramine 38.6mg/kg body wt)

Group (n=6)	Normal Sperms/1000	Abnormal Sperms/1000	% Of Abnormal Sperms
	Mean		
Control	980.7	19.33	1.93%
Imipramine 38.6mg/kg bodywt (¾ LD50)	932.5	67.6***	67.6%
Vitamin E 100 mg/kg+ Imipramine 38.6mg/kg body Wt	945.6	54.5	5.45%

P<0.05* P<0.01** P<0.001*** as compared to control



Graph 1: Percentage of abnormal sperms
(Control, Imipramine 12.9mg/kg body wt (¼LD$_{50}$), Vitamin E + Imipramine (12.9mg/kg body)



Graph 2: Percentage of abnormal sperms
Control, imipramine 25.8mg/kg body wt (½LD50) , Vitamin E+ Imipramine (25.8mg/kg body wt)

Figure 1: Normal Sperms

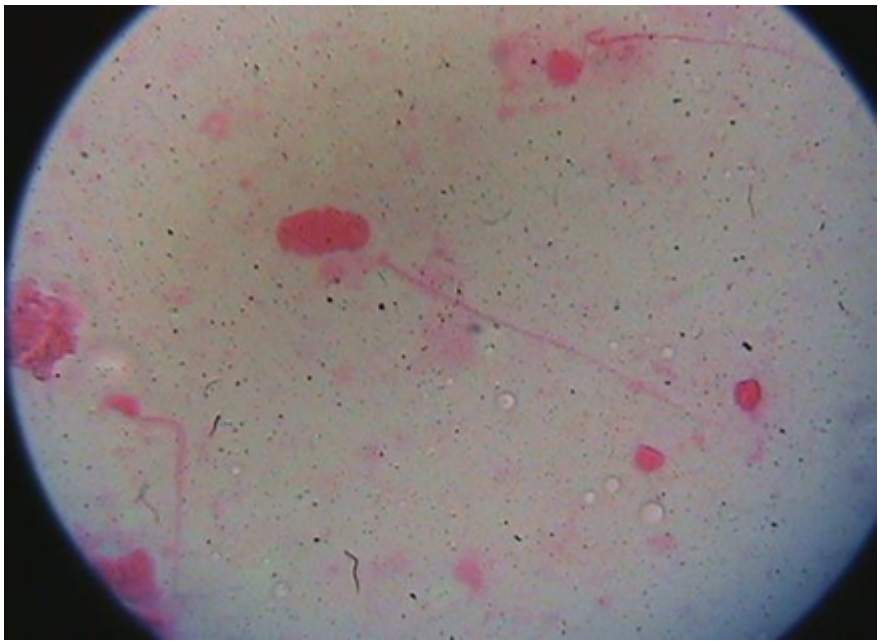


Figure 2: Abnormal sperm

Results

The present study examines the genotoxicity of imipramine in comparison to a control group and how vitamin E affects imipramine's genotoxicity in male albino mice.

One method is used to conduct the study: Sperm head abnormality: In this experiment, the prevalence of aberrant sperms in imipramine-treated and imipramine-plus-Vitamin E-treated groups was compared.

Test for sperm head abnormality: The dosages used in this procedure were based on the omeprazole LD50, which is 1/4 LD_{50} (1000 mg/kg body weight), 1/2 LD_{50} (2000 mg/kg body weight) and 3/4 LD_{50} (3000 mg/kg body weight), as well as vitamin E (100 mg/kg body weight).

The following Groups' mean incidence of abnormal sperm is shown in Table-IV:

Group I: Control -19.33
Group II: Imipramine 12.9mg/kg body wt (¼ LD_{50}) - 39.5
Group III: Imipramine 25.9mg/kg body wt (½ LD_{50}) - 48.16
Group IV: Imipramine 38.6mg/kg body wt (¾ LD_{50}) – 67
Group V: Vitamin E 100mg/kg body wt + Imipramine 12.9mg/kg body wt -20.5
Group VI: Vitamin E 100mg/kg body wt + Imipramine 25.8 mg/kg body wt – 34
Group VII: Vitamin E 100mg/kg body wt + Imipramine 38.6 mg/kg body wt- 54.33

The mean incidence of abnormal sperms were increased with the increasing dose of Imipramine in the Groups II, III and IV compared to control and was found statistically significant. The mean incidence of abnormal sperms was decreased only in the Group-V but not in Groups VI and VII when compared to Groups II, III and IV and found statistically significant only in Group V but not in other Groups.

Discussion

Drugs contribute to one of the major to which man has been exposed. Beside their use in therapy, they also produced side-effects which may affect the human health directly or indirectly including their genetic damage. Genetic toxicology is the study of the substances that can damage the DNA and chromosomes of the cell. This damage is usually measured as mutations, chromosome aberrations, DNA strand brakes or interference of repair of damage[11] Mutation is defined as a DNA sequence chain that leads to inheritable alteration of gene functions. Agents that change DNA sequence are toxic to the gene and thus designated as genotoxic because mutations are often associated with cancer and birth defects, the two most common fearsome human diseases, the genotoxicity of a commercial and an environmental chemicals is critical information for regulatory agencies for the assessment of human risks[12]. The methods for measuring the genotoxicity are numerous and various test system range from microorganism to mammals. Mice are being widely used for such studies[13]. The effective and simple tests appear to be micronucleus test and sperm head abnormality assay. As there are conflicting reports on mutagenicity of imipramine[14] and protective role of Vitamin E have been published, therefore the present study was conducted to obtain information on mutagenicity of Imipramine and protective role of Vitamin E. Both the drugs Imipramine and vitamin E were given orally.

Group I-Control received distilled water orally
Group II –Imipramine 12.9 mg/kg body wt (¼ LD_{50})
Group III-Imipramine 25.8mg/kg body wt (½ LD_{50})

Group IV-Imipramine 38.6 mg/kg body wt (¾ LD $_{50}$)
Group V- Vitamin E 100 mg/kg body wt + Imipramine 12.9mg/kg body wt
Group VI- Vitamin E 100 mg/kg body wt + Imipramine 25.8 mg/kg body wt
Group VII-Vitamin E 100 mg/kg body wt + Imipramine 38.6 mg/kg body wt

Sperm head abnormality assay: The sperm head abnormality assay was chosen for assessment of mutagenicity of physical and chemical agents as it provides a way of assessing the damage to the mammalian germ cells. It has been proposed by Wyrobek *et al* (1978)[15]. The development of abnormal sperm head morphology and variations in DNA content of spermatozoa are often genetically controlled. The occurrence of sperm head abnormalities have also been attributed to the chromosomal aberrations that occur during the packing of genetic material in the sperm head or occurrence of point mutation in testicular DNA. It may also arise as a consequence of naturally occurring level of mistakes in the spermatozoa differentiating process during spermatogenesis[16,17].

The results of present study showed that there was an increase in the incidence of abnormal sperms with increasing doses of Imipramine i.e. ¼LD50, ½LD50 & ¾LD50 in Groups II, III and IV in comparison to Group-I it means that Imipramine is genotoxic for all the mentioned doses in mice and was found statistically significant, the similar study done by K. K. Vijayalaxmi *et al*. on carboplatin with three doses i.e.30, 60, 90mg/kg body wt and carboplatin induced high frequency of abnormal sperms at higher doses and the effect was significant at all the three doses[18].

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