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Evaluation of genotoxicity of gastric proton pump inhibitor omeprazole and role of vitamin-E in mice

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Abstract
Objective: To evaluate the genotoxicity of gastric proton pump inhibitor omeprazole and role of vitamin-E in mice.
Materials and Methods: Forty two male albino mice were randomly divided into seven groups (n=6).Group I-control group which was served with distill water. In groups (II-IV) mice were treated with omeprazole i.e., 1000(1/4LD_{50}), 2000(1/2LD_{50}) and 3000(3/4LD_{50})mg/kg body wt orally for two days in two divided doses. Estimation of role of vitamin E in genotoxicity was done in groups (V, VI, VII) & Mice were treated initially with vitamin E with 100mg/kg body wt orally per day for six days, then omeprazole was given for two days in two divided doses(1/4, 1/2, and ¾ LD_{50}) after 6hrs of the last dose of omeprazole animals in all groups were sacrificed by cervical dislocation and bone marrow cells were aspirated, smear was made and staining was done, screened under microscope for the presence of micronuclei in bone marrow cells.
Results: The results were obtained by applying student's t-test. In the present study with micronucleus assay genotoxic effect of omeprazole with ¼, 1/2 ¾ LD_{50} doses was observed by the increase in the incidence of micronuclei in polychromatic and total erythrocytes in groups-II III and IV than group I and protective effect of vitamin E was observed by decrease in the incidence of micronuclei in polychromatic and total erythrocytes comparison to groups II, III and IV with the doses of omeprazole +vitamin E (100mg/kg body wt).
Conclusion: Omeprazole is having the genotoxicity effect on bone marrow cells with all the 3doses ¼, ½ and ¾ LD_{50} and vitamin E has got the protective effect for all the doses of omeprazole on the bone marrow.

Keywords: Omeprazole, genotoxicity, micronuclei, vitamin-E, polychromatic erythrocytes

Introduction
Peptic ulcer is one of the common gastrointestinal disease for which most commonly used drugs are the proton pump inhibitors like omeprazole which is available as over the counter drugs. A few reports are available on the mutagenic potential of these drugs. It is clinically important from the safety evaluation point of view to evaluate mutagenicity because millions of patients are treated with these drugs over a long period of time. The majority of ulcer will heal in 4-6 weeks of treatment. The prevention of relapse can be achieved only with the maintenance of treatment for years [1]. It is well known fact that increase in mutation may pose greatest implication on the possible production of genetic disaster to the future generation. Genetic damage can accelerate the process of ageing cause cancer and give rise to a variety of genetic diseases and pathological disorders. Antioxidants like Vitamin E, A, C and carotenoids are known to be reducing agents and these are the molecules capable of preventing the oxidation of other molecules. They terminate the oxidative chain reactions by removing free radicals. hence a lot of previous scientific reports have clearly documented the anti genotoxic potential of these antioxidants [2]. In view of the above mentioned importance the present work has been done. i.e., to see the genotoxicity of omeprazole and role of vitamin-E.

Materials and Methods
Materials
I) Animals
Eighty four male Swiss albino mice weighing 25 to 30 grams aged 8 to 9 weeks were obtained from the animal house, Department of Pharmacology. All the animals were maintained under the standard condition with proper feed and water ad libitum in the animal house as per Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines. The study had been approved by the Institutional Animal Ethical Committee (IAEC) before the experimental study.

II) Drugs

a) Omeprazole

In the present study omeprazole which was available in the powdered form in a vial of strength of 40 mg/10ml from DR REDDYS Laboratory Ltd. The drug was made to dissolved in 1ml of distilled water to make 40mg/ml. the doses in the present study was selected on the basis of LD_{50} of the omeprazole in the mice -4gms/kg body wt [3]

b) Vitamin E

In the present study the vitamin E which is available as paediatric drops of strength of 50mg/ml from MERK LTD was used. The dose used in the present study is 100 mg /kg body wt and was administer oral route of administration [4, 5]

Electron microscope:-Electron microscope was used for screening of the slides under oil immersion.

Giemsa stain: It is Romanowsky stain which is used for staining the bone marrow aspirate smeared slides.

Human Serum: It is used to prevent the clumping of the bone marrow cells while aspiration.

0.9% Saline: It is used to keept the epididymis and femur of mice in petridish.

Feeding tube: used for oral feeding of mice.

In the present study the following methods were employed to evaluate the genotoxicity of omeprazole and role of vitamin E in the prevention of genotoxicity are

Micronucleus Test [6-9]: Micronucleus assay has been widely used to measure genotoxicity both *in vitro* and *in vivo*, accordingly the test was designed by schmid (1973). The objective of the test is to evaluate a test substance for clastogenic activity in polychromatic erythrocytes cells in mice. Forty two male albino mice weighing between 25-30gms were selected for the study. They were fasted over night with water ad libitum prior to the day of experiment. The mice were divided into seven groups of six mice each.

Group I	-control received distilled water
Group II	- omeprazole1000mg/kgbodywt(¼ LD_{50})
Group III	- omeprazole 2000mg/kg body wt(½ LD_{50})
Group IV	- omeprazole3000mg/kg body wt (¾ LD_{50})
Group V	-Vitamin E 100mg/kg bodywt + omeprazole 1000mg/kg body wt
Group VI	- Vitamin E 100mg/kg body wt+ omeprazole 2000mg/kg body wt,
Group VII	-vitamin E 100 mg/kg body wt + omeprazole3000mg/kgbodywt

In Control Group mice received 0.5 ml of distilled water orally. In Groups II, III and IV the total calculated dose of the omeprazole was given orally for two days in two divided doses then six hours after the last dose of omeprazole, animals in all the groups sacrificed by cervical dislocation where as in Groups V, VI and VII initially the animals received Vitamin-E 100mg/kg body wt per day orally for six days, then omeprazole was given orally for two days in two divided doses, after six hours of the last dose of the omeprazole, animals in all the groups were sacrificed by cervical dislocation.

Immediately after sacrificing the animals, the femora from the mice were removed and the proximal end of the femur was carefully cut then a 22 gauge needle was mounted on tuberculin syringe and was inserted in the proximal opening of the femora and bone marrow cells were aspirated and placed on the slide and a small drop of human serum was added and homogenous preparation made. Viscous suspension was dropped on a clear slide and smear was allowed to dry and stained

Staining Procedure

The slides were stained within 24 hours after making the smear to get the best results which are as follows:

1. The slides were mixed in absolute methanol for 5 minutes.
2. Washed in distilled water in order to remove methanol and allowed to dry.
3. The dried slides were stained with giemsa and distilled water for 10 minutes.
4. The slides were washed in distilled water dried and mounted using Euparol. The smear then prepared was systematically screened under 100X magnification for the presence of normochromatic and polychromatic cells with micronuclei. The ratio of polychromatic to normochromatic cells were utilized to determine the effect of drug on the activity of proliferative bone marrow cells. About 3000 immature blue polychromatic erythrocytes (PCE) and corresponding mature pink normochromatic erythrocytes cells(NCE)were scored per animal and was utilized to determine the effect of drug on the proliferative activity of the bone marrow the data was further subjected to statistical analysis by student's't' test.

Results

The present study was based on the evaluation of genotoxicity of omeprazole in comparison to control and how vitamin E is playing the role in genotoxicity in comparison to omeprazole in male albino mice.

The study was carried out by one method

Micronucleus test: In this test the incidence of micronuclei in polychromatic, normochromatic erythrocytes of Omeprazole compared with Control and Omeprazole+ Vitamin E compared with Omeprazole.

Micronucleus test

The doses administered in this method were based on LD_{50} of omeprazole i.e. ¼ LD_{50} (1000mg/kg body wt), ½ LD^{50} (2000mg/kg body wt), ¾ LD^{50} (3000mg/kg body wt) and vitamin E (100mg/kg body wt)

Group I: Control

The mean incidence of micronuclei in polychromatic erythrocytes/3000cells is-13(table-1), in normochromatic erythrocytes is-6.8(table-2) and in total erythrocytes is-19.3(table-3).

Group II: Omeprazole 1000mg/kg body wt (¼LD_{50})

The mean incidence of micronuclei in polychromatic erythrocytes/3000cells is-25.16 (Table-1),in normochromatic erythrocytes is-8.6(table-2) and in total erythrocytes is-33.83(Table-3)

Group III: Omeprazole 2000mg/kg body wt (½LD_{50})

The mean incidence of micronuclei in polychromatic erythrocytes/3000cells is -35.3(Table-1), in normochromatic erythrocytes is-7.8(Table-2) and in total erythrocytes is-33.83 (Table-3).

Group IV: Omeprazole 3000mg / kg body wt (¾LD$_{50}$)
The mean incidence of micronuclei in polychromatic erythrocytes/3000 cells is-43.16 (Table-1), in normochromatic erythrocytes is -7.83(Table-2) and in total erythrocytes is-51(Table-3). -7.83(Table-2) andintotalerythrocytesis-51(Table-3)

Group V: vitamin E 100mg/kg body wt + Omeprazole 1000mg/kg body the mean incidence of micronuclei in polychromatic erythrocytes/3000cells is-12.6(Table-1), in normochromatic erythrocytes is-8.3(Table-2) and in total erythrocytes is-20.5(Table3).

Group VI: Vitamin E 100mg/kg body wt + omeprazole 2000 mg/kg body wt
The mean incidence of micronuclei in polychromatic erythrocytes is-20.16(Table-1), in normochromatic erythrocytes is-7.5(Table-2) and in total erythrocytes is-30.8(Table-3).

Group VII: Vitamin E 100mg/kg body wt + Omeprazole 3000mg/kg body wt. The mean incidence of micronuclei in polychromatic erythrocytes/3000cells is -29 (Table-1), in normochromatic erythrocytes is-8(Table-2) and in total erythrocytes is-37(Table-3).

The mean incidence of micronuclei in polychromatic and total erythrocytes /3000 cells were gradually increased with increasing the dose of omeprazole in Groups II, III and IV in comparison to Group I and found statistically significant, whereas mean incidence of micronuclei in normochromatic erythrocytes with increasing the dose of omeprazole in Groups II, III and IV in comparison to Group I found to be statistically insignificant.
The mean incidence of micronuclei in polychromatic and total erythrocyte s/3000cells were gradually decreased with the doses of omeprazole+ vitamin E in Groups V, VI and VII in comparison to Groups II, III and IV found statistically significant, whereas mean incidence of micronuclei in normochromatic erythrocytes with doses of omeprazole + Vitamin E in Groups V, VI and VII in comparison to Groups II, III and IV found to be statistically insignificant. The ratio between polychromatic to normochromatic erythrocytes ratio (P/N ratio) in Group I- 1(Table-IV), GroupII-1 (Table-IV), GroupIII-1 (Table-V), GroupIV-1 (Table-VI), GroupV-1 (Table IV),
Group VI-1 (Table-V) and Group-VII-1 (Table VI). It shows that the ratio of polychromatic to normochromatic erythrocytes was not differing from that of control in any groups indicating that the drug has not induced an bone marrow depletion.

Table I: Micronucleus Test Incidence of Micronuclei in Polychromatic Erythrocytes (PER 3000 CELLS)

S. No	Control Group	Omeprazole ¼ LD$_{50}$	Omeprazole ½ LD$_{50}$	Omeprazole ¾ LD$_{50}$	Vitamin–E + 100 mg /kg body weight	Vitamin–E + 100 mg /kg body weight	Vitamin–E + 100 mg /kg body weight
Mice		1000 mg /kg body weight	2000 mg /kg body weight	3000 mg /kg body weight	Omeprazole ¼ LD$_{50}$	Omeprazole ½ LD$_{50}$	Omeprazole ¾ LD$_{50}$
1	14	24	35	45	12	22	33
2	12	21	38	42	14	20	30
3	12	30	32	48	13	25	27
4	15	28	40	41	11	24	30
5	11	22	31	43	10	26	28
6	14	26	36	40	13	22	26
Mean	13	25.16	35.33	43.16	12.16	20.16	29
SD	1.54	3.48	3.44	2.92	1.47	3.98	2.52
SE	0.631	1.42	1.40	1.19	0.602	1.631	1.032

Table II: Micronucleus Test

S. No	Control Group	Omeprazole ¼ LD$_{50}$	Omeprazole ½ LD$_{50}$	Omeprazole ¾ LD$_{50}$	Vitamin–E + 100mg/kg body weight	Vitamin-E 100 mg /kg body weight+	Vitamin-E 100mg /kg body weight+
Mice		1000 mg /kg body weight	2000 mg /kg body weight	3000mg /kg body weight	Omeprazole ¼ LD$_{50}$	Omeprazole ½ LD$_{50}$	Omeprazole LD$_{50}$ 3/4th
1	7	8	8	8	8	10	10
2	8	10	9	6	10	6	9
3	5	12	6	9	9	8	8
4	7	7	7	8	6	7	6
5	8	6	8	9	7	5	10
6	6	9	9	7	10	9	5
Mean	6.8	8.6	7.8	7.83	8.3	7.5	8
SD	1.16	2.16	0.876	1.077	2.76	1.87	1.78
SE	0.475	0.88	0.359	0.44	1.131	0.766	0.729

Table III: Micronucleus Test Incidence of Micronuclei in Total Erythrocytes (Per 3000 Cells)

S. No	Control Group	Omeprazole ¼ LD^{50}	Omeprazole ½ LD^{50}	Omeprazole ¾ LD^{50}	Vitamin–E + 100 mg /kg body weight	Vitamin–E + 100 mg /kg body weight	Vitamin–E + 100 mg /kg bodyweight
Mice		1000 mg /kg body weight	2000 mg /kg body weight	3000 mg /kg body weight	Omeprazole ¼ LD$_{50}$	Omeprazole ½ LD$_{50}$	Omeprazole ¾ LD$_{50}$
1	21	32	43	53	20	32	43
2	20	31	47	48	24	26	39
3	17	42	38	57	22	33	35
4	22	35	47	49	17	32	36
5	19	28	39	52	17	31	38
6	30	35	45	47	23	31	31
mean	19.83	33.83	43.16	51	20.5	30.8	37
SD	1.16	4.791	3.91	3.66	2.57	2.48	3.97
SE	0.475	1.96	1.60	1.5	1.05	1.01	1.62

Table IV: Micronucleus Test (Control, Omeprazole 1000mg/kg body wt, VITAMIN E + OMEPRAZOLE 1000mg/kg body wt)

Group (n=6)	Micronuclei In PCE/3000 CELLS	Micronuclei In NCES / 3000 CELLS	Micro Nuclei In Total Erythrocytes	P/N ratio
	Mean	Mean	Mean	
control	13 (0.43)	6.8 (0.22)	19.8 (3.3)	1
Ome1000mg/kg bodywt	25.16 (0.83)***	8.6 (0.28)	33.83 (5.63)***	1
body (¼ LD_{50})				
Vitamin E (100 mg/kg)+	12.16 (0.40) $^{\$\$}$		20.5 (3.41)$^{\$\$}$	
Omeprazole 1000 mg/kg		8.3 (0.27)		1
body wt				

Values shown in parenthesis indicate percentage
P<0.05* *P*<0.01** *P*<0.001*** as compared to control
P<0.05$^{\$}$*P*<0.01$^{\$\$}$*P*<0.001$^{\$\$\$}$as compared to Omeprazole

Table V: Micronucleus Test (Control, Omeprazole 2000mg/kg body wt, VITAMIN E + OMEPRAZOLE2000mg/kg body wt

Group (n=6)	Micronuclei In PCE/3000 CELLS	Micronuclei In NCE / 3000 CELLS	Micro Nuclei In Total Erythrocytes	P/N ratio
	Mean	Mean	Mean	
Control	13 (0.43)	6.8 (0.22)	19.8 (3.3)	1
Omeprazole2000mg/kg	35.33 (1.17)***	7.8 (0.26)	43.16 (7.19)***	1
body wt (½ LD_{50})				
Vitamin E 100 mg/kg+	20.16 (0.67)$^{\$\$}$		30.8 (4.61)$^{\$\$}$	
Omeprazole 2000 mg/kg		7.5 (0.25)		1
body wt				

Values shown in parenthesis indicate percentage micronuclei
P<0.05* *P*<0.01** *P*<0.001*** as compared to control
P<0.05$^{\$}$*P*<0.01$^{\$\$}$*P*<0.001$^{\$\$\$}$as compared to Omeprazole

Table VI: Micronucleus Test (Control, Omeprazole 3000mg/kg body wt, VITAMIN E + OMEPRAZOLE 3000mg/kg body wt)

Group (n=6)	Group(n=6) PCE/3000 CELLS	Micronuclei In NCE/ 3000 CELLS	Micro Nuclei In Total Erythrocytes	P/N ratio
	Mean	Mean	Mean	
Control	13 (0.43)	6.8 (0.22)	19.8 (3.3)	1
Omeprazole 3000mg/kg	43.16 (1.43)***	7.83 (0.26)	51 (8.5)***	1
body wt (¾ LD_{50})				
Vitamin E 100 mg/kg+	29 (0.96)$^{\$\$}$		37 (6.16)$^{\$\$}$	
Omeprazole 3000 mg/ kg		8 (0.26)		1
body wt				

Values shown in parenthesis indicate percentage micronuclei
P<0.05* *P*<0.01** *P*<0.001*** as compared to control
P<0.05$^{\$}$*P*<0.01$^{\$\$}$*P*<0.001$^{\$\$\$}$ compared to Omeprazole

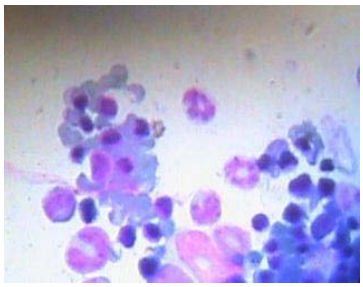

Fig 1: normal bone marrow cells

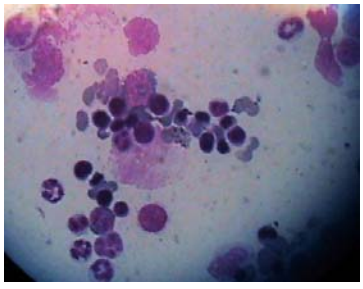

Fig 2: Micronuclei (abnormal) in bone marrow cells

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 [10]. The regulatory genetic toxicology tests are a series of well-defined mutagenicity test designed to detect chemical and physical agents capable of inducing mutations.

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 [11].

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 [12]

The effective and simple tests appear to be micronucleus test and sperm head abnormality assay.

As there are conflicting reports on mutagenicity of Omeprazole [13, 14] and protective role of Vitamin E have been published, therefore the present study was conducted to obtain information on mutagenicity of Omeprazole and protective role of Vitamin E.

The animals were divided into seven groups of six mice each for both tests. Both the drugs omeprazole and vitamin E were given orally

Group I	-Control received distilled water orally
Group II	-Omeprazole1000 mg/kg body wt (1/4 LD_{50})
Group III	-Omeprazole 2000 mg/kg body wt (1/2 LD_{50})
Group IV	-Omeprazole3000 mg/kg body wt (3/4 LD_{50})
Group V	- Vitamin E 100 mg/kg body wt + omeprazole 1000 mg/kg body wt,
Group VI	-Vitamin E 100 mg/kg body wt + omeprazole 2000 mg/kg body wt,
Group VII	-Vitamin E 100 mg/kg body wt + omeprazole 3000 mg/kg body wt

Micronucleus assay

The micronucleus assay has been widely used to measure genotoxicity both in-vitro and in-vivo. This method is designed by Schmid (1973).The micronucleus assay is devised primarily for evaluating the ability of the test substances to induce structural and chromosomal damage. Both kinds of damages are associated with the appearance or progression of tumors and with adverse reproductive and developmental outcome. The micronucleus formation may be due to free radical generation from an agent leading to lipid per-oxidation of membrane causing the breakages of the DNA and covalently binding between the products of lipid peroxidation [15].

An increase in the proportion of micronucleated polychromatic erythrocytes with test substance is an indication of chromosomal damage induction [16]. The results in the present study showed there was increase in micronuclei in polychromatic erythrocytes in Groups II, III and IV in comparison to Group I. This indicates that Omeprazole is genotoxic for all the three mentioned doses i.e. ¼ LD_{50}, ½LD_{50} ¾LD_{50}, the mechanism of genotoxicity is unclear. The following could be one the mechanisms; Omeprazole is a potential genotoxic carcinogen probably due to unstable sulphenamide metabolite [17].

In Groups V, VI and VII there was decrease in the incidence of micronuclei in polychromatic erythrocytes when compared to Groups II, III and IV. This indicates that vitamin E has protective effect against omeprazole induced genotoxicity for all the three mentioned doses in mice and was found statistically significant.

Vitamin E, a fat soluble vitamin, has antioxidant property and prevents damage induced by free radicals generated by normal metabolic processes and external substances. Vitamin E reacts with the lipid peroxide radicals formed by per oxidation of polyunsaturated fatty acids before they can establish a chain reaction, acting as a free radical trapping antioxidant [18].There was few reports showing that Vitamin E is genoprotective against Rifampicin with its antioxidant property [2].

Vitamin E has also been documented to be protective against chromosomal aberrations and mutation induced by Sodium chromate in Chinese hamsters in V 79 cells [19]. Similarly Vitamin E has genoprotective role against ome.

Conclusion

The conclusion of the present study is that omeprazole has genotoxicity effect on all the three mentioned doses. i.e. ¼LD_{50}, ½LD_{50} and ¾LD_{50} as seen in micronucleus test in male mice. Vitamin E has genoprotective for all the three above mentioned doses of omeprazole in micronucleus test.

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