

Research Article**Protective effect of marketed herbal formulation on the mutagenicity of Omeprazole in mice**Shiny Ganji¹, A. M. Ismail², Easwari Pasam^{3*}, B. Swathi³¹St. Ann's College of Pharmacy, Chirala, Prakasam district, Andhra Pradesh, India²Periyar College of Pharmaceutical Sciences, Periyar Centenary Educational Complex, Tiruchirappalli, Tamil Nadu 620021 India³Priyadarshini College of Pharmaceutical Education and Research, Guntur, Andhra Pradesh, India

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Abstract

Objective: The objective of the study is to evaluate the protective effect of a herbal formulation on the mutagenic effect of Omeprazole in mice. **Materials and methods:** Four groups of adult Swiss mice (6-8 weeks old) weighing 18-22 gm (n = 6) were used. The normal control group received saline and test group –I animals were treated with Omeprazole (25 mg/mouse, p.o.) to sub group-1 and (50 mg/mouse. p.o.) to sub group-II for five days. To test group - II Omeprazole is administered along vitamin E (50 mg/kg, p.o.) for a period of 35 days. Test group-III has received herbal formulation (1.3 mg/mouse, p.o.) for 35 days. On 35th day the animals were sacrificed by cervical dislocation and sperm head abnormality assay was performed. **Results:** Omeprazole which was administered in lethal dose has showed a dose dependent mean increase in sperm abnormalities. Omeprazole with vitamin E showed that there is significant decrease in the percentage of abnormal sperm in lower doses of Omeprazole i.e. 25 mg than with 50 mg dose. A similar protection was observed in the group treated with herbal formulation challenged by Omeprazole. **Conclusion:** Omeprazole is capable of inducing abnormalities in mice in a dose dependent manner perhaps of its unscheduled DNA synthesis. Vitamin E offered protection on the sperm abnormalities of mice due to its anti-oxidant activity. Herbal formulation has also showed a better protection on the mutagenic effect of Omeprazole in mice as compared to that of vitamin E.

Keywords: Mutagenesis, Omeprazole, vitamin E, sperm head abnormality

Introduction

Drugs on one hand have utility with curative properties and on other hand may pose some hazards to the human health directly or indirectly. Mutational changes occur in both somatic and germ cells (Mohsin et al., 2011). Mice treated with chemical mutagens and radiations have an increased incidence of morphologically abnormal spermatozoa (Fabricant et al., 1981). Life style behaviors cause destructive effects on various cellular organelles like mitochondria, sperm DNA etc (Agarwal et al., 2005).

Omeprazole has the ability to induce unscheduled DNA

synthesis in the gastric mucosa which is an indicator of genotoxicity (Furihata et al., 1991). Since sperms are produced continually from puberty, exposure to genotoxic effects may occur throughout adult life (Hewak 2000). Omeprazole-a gastric proton pump inhibitor used in the treatment of peptic ulcer has been tested for its mutagenicity in germ cells of mice by sperm-head abnormality assay; showed a dose dependent mean increase of sperm abnormalities (Mohsin et al., 2011). The sperm abnormality assay is one of the newly introduced tests in the protocols of mutagenicity screening programmes (Goud et al., 1982).

Antioxidants are one of the most important components to having healthy fertility that every man and women needs to focus on. They help to protect the sperm from free radical damage. Free radicals are able to damage both sperm cell health and the cell's DNA. Studies have also shown a connection between oxidative stress caused from free

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radicals and male infertility. Tocopherol has been shown in studies to improve sperm health and motility in men. Studies have shown a diet deficient in Vitamin E to be a cause of infertility in rats. Optimum dose of vitamin E reverses free radical-mediated oxidative damage on motility, viability and Lipid peroxidation of bull's sperm (Bansal et al., 2009).

Herbal formulations greatly work in a faint manner that unites with the flow of the body's healing processes. Many herbal preparations are claimed to possess activities of rejuvenation and revitalization. The particular formulation of this study (ACTON[®]) is a well-known preparation used for treating general debility by the famous Ayurvedic hospital Ashtanga Ayurveda located in Kerala and is present in Trichy. Acton consists of plants such as *Curculigo orchoides*, *Pueraria tuberosa*, *Prunus dulcis*, *Orchis latifolia*, *Mucuna pruriens*, *Nilgiranthus cillatus*, *Meremmia tridentate*, *Sida rhombifolia*, *Tinospora cordifolia*, *Asparagus racemosus*, *Bacopa monnieri*, *Withania somnifera*.

Materials and methods

Formulation and other chemicals

ACTON formulation is collected from famous Ayurvedic hospital Ashtanga Ayurveda located in Kerala and is present in Trichy. Omeprazole is a kind of gift sample obtained from tablets (India) Limited, Chennai. Eosin Y is obtained from Madras Scientific Supplies.

Experimental animals

Male adult Swiss-A mice (*Mus musculus*) weighing 24-27 gm were used for Sperm head abnormality assay. The mean age was 6-8 weeks. The animals were selected at random and grouped into six (unless otherwise specified) and were acclimatized to our laboratory conditions for at least 3 days before induction into experiments. Each group, housed in polypropylene cages provided with husk bedding was fed with standard lab feed pellets supplied by Amrut Pranav Ltd., Bangalore and fresh filtered tap water *ad libitum*. The house temperature was maintained at 25±2°C with relative humidity of 50-70% and 12 hours light/Dark cycles. The Institutional Animal Ethics Committee of the Periyar college of Pharmaceutical Sciences, Trichy set up under the observance of the Committee for the Purpose of Control and Supervision on Experimental Animals (Reg.No.265/CPCSEA) approved all experimental procedures (PCP/IAEC/003/2012).

Experimental protocol

The mice were divided into 4 groups. Control group consisted of six animals. Remaining three groups were divided into two sub groups. Each sub group was having six animals each.

Group 1: Control group (Distilled water, 10 ml/kg, *p.o.*)

Group 2: Omeprazole

Sub group 1- Omeprazole (25 mg/mouse, *p.o.*)

Sub group 2- Omeprazole (50 mg/mouse, *p.o.*)

Group 3: Omeprazole and Vitamin E.

Sub group 1: Omeprazole (25 mg/mouse, *p.o.*)+Vitamin E (50 mg/kg, *p.o.*)

Sub group 2: Omeprazole (50 mg/mouse, *p.o.*)+Vitamin E (50 mg/kg, *p.o.*)

Group 4: Omeprazole and Herbal formulation

Sub group 1: Omeprazole (25 mg/mouse, *p.o.*) + Herbal Formulation (1.3 mg/mouse, *p.o.*)

Sub group 2: Omeprazole (50 mg/mouse, *p.o.*) + Herbal formulation (1.3 mg/mouse, *p.o.*)

Male Swiss mice aged 6-8 weeks weighing 24-27 gm were used. They were maintained with standard diet in an air-conditioned room of the Animal house of the College. In the sperm head abnormality assay, the total amount of the omeprazole was given orally for five days. The first group was given distilled water which acted as sham control. The second group consisted of 2 subgroups having 6 animals in each set. The two subgroups of animals were given ¼, ½ LD₅₀ dose (25, 50 mg / mouse) of Omeprazole for 5 days. The third group was similarly divided as the second group and was given the same dose of Omeprazole. In addition to Omeprazole, vitamin E was given in dose of 50 mg/kg body weight for a period of 35 days. To the fourth group Omeprazole was given along with Herbal drug formulation (1.3 mg/mouse) for a period of 35 days. All the animals were sacrificed by cervical dislocation on 35th day and epididymal sperm samples were collected from each animal separately.

Sperm head abnormality assay

Spermatogenesis in mice takes about five weeks (35 days) to complete according to (Hedde et al., 1979). The caput and cauda epididymis excised from the male mice were placed in a petri dish containing 1ml of physiological saline and then minced and teased carefully well with fine scissors and forceps to release the spermatozoa. After gentle pipetting, the suspension was separated from the tissue fragments and to this suspension was added a drop of 1% Eosin Y solution (10: 1) for 30 minutes. Air-dried smears were prepared on clean, grease-free glass slides using another clean slide angularly positioned at 300 to spread the drop through the whole length of the slide. The slides were then coded, randomized and examined cytologically under 40x binocular light microscopy. The aberrant ones were later photographed with a photomicroscope at 800x magnifications (Aduloju et al., 2008).

Statistical analysis

All the recorded results are expressed as mean \pm SEM (n=6). Statistical difference in mean was analyzed by using two-way ANOVA (analysis of variance); ***P<0.0001 vs +ve control which were considered as statistically significant.

Results

Omeprazole (25 mg) induced sperm abnormalities

Results of this study showed that the 250 number of sperms observed, mean maximum number of abnormal sperms were found to be in Omeprazole treated group (25.167 \pm 4.490), least number of abnormalities were observed in Omeprazole + Vitamin E treated group (12.833 \pm 0.401) and next lower number has been observed in Omeprazole + Herbal Formulation treated group (15.833 \pm 1.138) (Table 1 & Figure 1).

Omeprazole (50 mg) induced sperm abnormalities

Result of the study showed 250 number of sperms were observed, mean maximum number of abnormal sperms were found to be in Omeprazole treated group (29.500 \pm 3.964), least number of abnormalities were observed in Omeprazole + Herbal Formulation treated group (12.600 \pm 2.249) and next lower number has been observed in Omeprazole + Vitamin E treated

group (14.333 \pm 0.955) (Table 2 & Figure 1).

Percentage of abnormal sperms at different doses of Omeprazole

Among the doses administered (25 & 50 mg) of Omeprazole, 50 mg dose produced maximum number of abnormal sperms in the Omeprazole only treated group as compared to that of the 25 mg of the dose. 25 mg Omeprazole + Vitamin E showed less number of abnormal sperms as compared to that of herbal formulation treated group of same dose. Similarly use of 50 mg of Omeprazole along with the herbal formulation treated group showed less number of abnormalities than that of Omeprazole + Vitamin E treated group of same category (Table 3 & Figure 2).

Protective effect of the herbal formulation on the mutagenic effect of Omeprazole in mice

Herbal formulation of 50 mg dose of Omeprazole showed greater protection (59.92%) than the vitamin E of the same dose whereas vitamin E showed better protection than herbal formulation in 25 mg dose of Omeprazole category (Table 4 & Figure 4). Various types of abnormal sperms can be observed in all groups of animals (Figure 3).

Table 1. Omeprazole (25mg) Induced Sperm Abnormalities

Groups	Normal sperms Count	Abnormal sperms count
Control	230.833 \pm 1.014	19.167 \pm 1.014
Omeprazole	224.833 \pm 4.490	25.167 \pm 4.490
Omeprazole + Vitamin E	237.167 \pm 0.401***	12.833 \pm 0.401***
Omeprazole + Herbal Formulation	232.500 \pm 1.232***	15.833 \pm 1.138***

Values as Mean \pm S.E.M (n=6). ***P<0.0001 vs +ve control by two way ANOVA

Table 2. Omeprazole (50mg) Induced Sperm Abnormalities

Groups	Normal sperms Count	Abnormal sperms count
Control	230.833 \pm 1.014	19.167 \pm 1.014
Omeprazole	220.500 \pm 3.964	29.500 \pm 3.964
Omeprazole + Vitamin E	235.667 \pm 0.955***	14.333 \pm 0.955***
Omeprazole + Herbal Formulation	236.167 \pm 2.056***	12.600 \pm 2.249***

Values as Mean \pm S.E.M (n=6). ***P<0.0001 vs + ve control by two way ANOVA

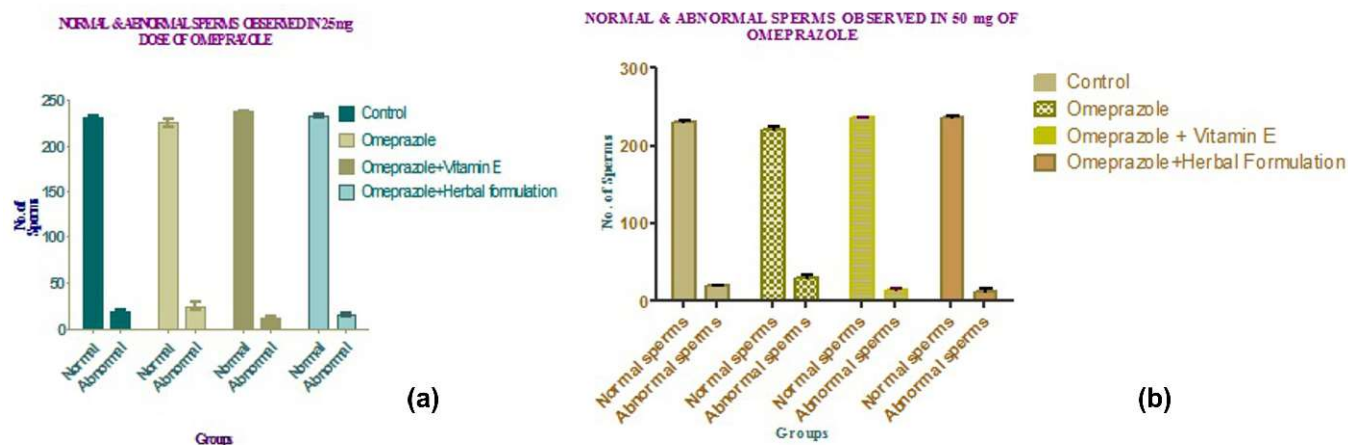


Figure 1. Omeprazole induced Sperm Abnormalities (a) Omeprazole-25 mg (b) Omeprazole-50 mg

Table 3. Percentage of abnormal sperms

Groups	% Abnormality	
	Omeprazole (25 mg)	Omeprazole (50 mg)
Control	8.303	8.303
Omeprazole	11.19	13.3
Omeprazole+ Vitamin E	5.41	6.08
Omeprazole+ Herbal Formulation	6.809	5.33

Table 4. Protective effect (%) of vitamin E and herbal formulation

Groups	% Protection	
	Omeprazole (25 mg)	Omeprazole (50 mg)
Omeprazole+ Vitamin E	51.65	54.2
Omeprazole+ Herbal Formulation	39.15	59.92

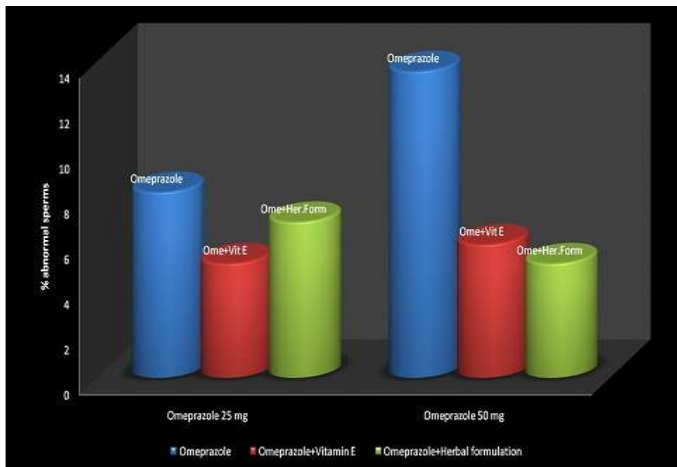


Figure 2. Percentage of Abnormal Sperm

% Protection offered by the herbal drug formulation as compared with vitamin E

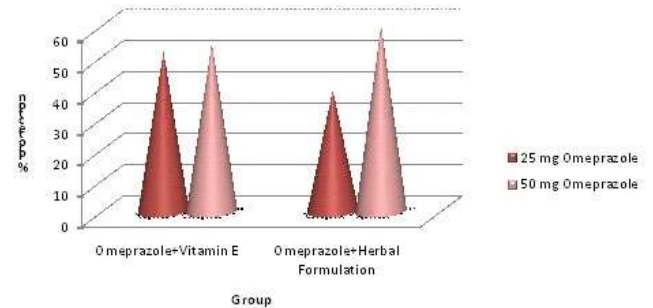


Figure 3. Percentage protection offered by the herbal drug formulation as compared with vitamin E

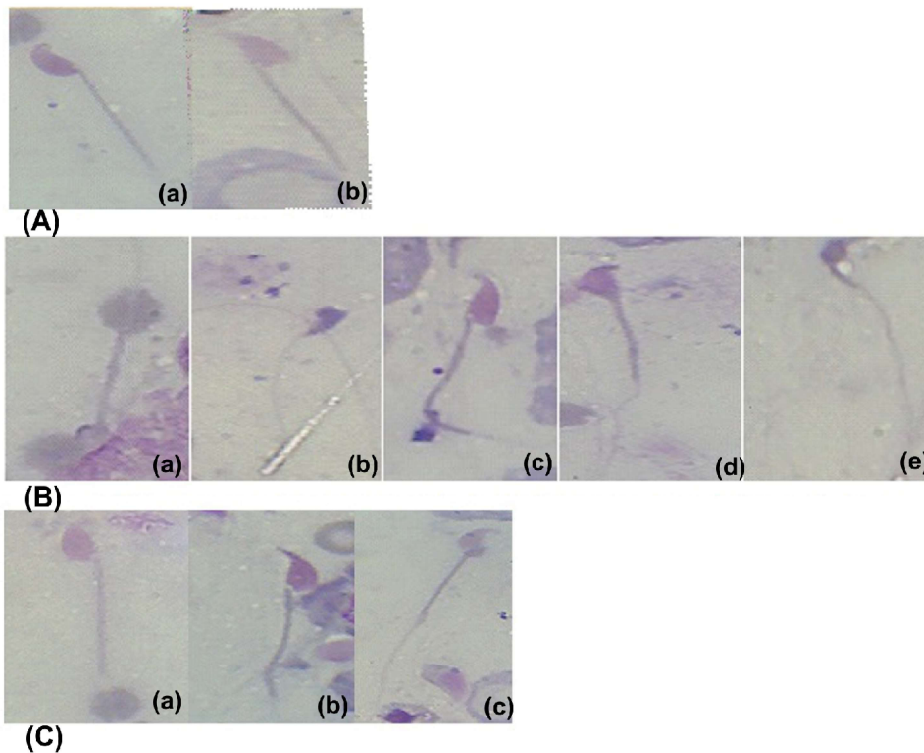


Figure 4. Different types of sperm observed in different groups: (A) Control group: (a) Sperm with hook at wrong angle (b) hook pointed upward; (B) Omeprazole treated group showed sperm with (a) amorphous head (b) Knobbed head (c) bent tail (d) two tails (e) pin head; (C) Formulation treated group showed sperm with (a) bent neck (b) hook at upward (c) normal sperm

Discussion

Omeprazole is one of the important proton pump inhibitor used in the treatment of peptic ulcer has been tested for its mutagenicity in germ cells of mice by sperm-head abnormality assay; showed an dose dependent mean increase of sperm abnormalities i.e., 25.167 ± 4.490 in 25 mg to a peak of 29.500 ± 3.964 in 50 mg. Mice treated with chemical mutagens and radiations have an increased incidence of morphologically abnormal spermatozoa. This incidence is dose-related and appears to be genetically inherited. The basis for these abnormal spermatozoa, while not completely understood, may be related to cellular changes caused by chemical carcinogens and/or ageing.

Increased incidence of DNA fragmentation in ejaculated spermatozoa can be reduced by oral treatment with two antioxidants, vitamins C and E. Studies have proven that vitamin E in optimum doses reverses free radical-mediated oxidative damage on motility, viability of bull's sperm (Bansal et al., 2009).

In our study we have supplemented omeprazole with vitamin E to see the effect on sperm morphology. Our results showed that there is significant decrease in the percentage of abnormal sperms in lower dose of omeprazole i.e. 25 mg than that with 50 mg dose (Mohsin et al., 2011).

A similar protection was observed in this study in the group treated with the herbal formulation challenged by omeprazole. The percentage of abnormalities observed in the study was lower in the group receiving 50 mg of Omeprazole (5.33%) and treated with the herbal formulation while maximum numbers of abnormalities were noted in the group of 50 mg of Omeprazole (13.3%) alone.

Lifestyle behaviours such as smoking and alcohol use and environmental pollution further enhance the generation of ROS and thus, cause destructive effects on various cellular organelles like mitochondria, sperm DNA (Agarwal et al., 2002).

Our herbal formulation showed protection lesser (39.15%) than that of vitamin E (51.65%) in 25 mg of Omeprazole treated group; whereas in 50 mg of Omeprazole treated group, it showed better protection (59.92%) than that of vitamin E (54.2%).

From this study it has been concluded that Omeprazole is capable of inducing sperm abnormalities in mice in a dose dependent manner (Mohsin et al., 2009). Vitamin E which has a well established anti-oxidant activity, offered protection on the sperm abnormalities of mice on treatment for a period of 35 days & it showed remarkable protection with increased doses of Omeprazole. The herbal formulation which has many plants as its key ingredients with well-known anti-oxidant activity, aphrodisiac nature and neuroprotective action showed a better action than that of vitamin E in the category of 50 mg dose of

Omeprazole. It showed an increase in protection on increased dose of Omeprazole.

Therefore we conclude the herbal formulation that has been undertaken for the study has the ability to reduce the sperm abnormalities perhaps due to its antioxidant activity. It showed a better protection on the mutagenic effect of Omeprazole in mice as compared to that of vitamin E.

Conflict of interest

None

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