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# EVALUATION OF THE POTENTIAL GENOTOXICITY AND DEVELOPMENTAL TOXICITY OF MANJARIX

Aida I El makawy[1]*, Ashraf B Abdel-Naim[2], Alaa Barakat3

[1]*Cell Biology Department, National Research Centre, 33 El Bohouth St., Dokki, Giza, Egypt-P.O.12622 [2]Department of Pharmacology and Toxicology, Faculty of Pharmacy, Ain Shams University, Cairo, Egypt.[3] Biochemistry and Biotechnology Department, Family of Pharmacy, Heliopolis University, Cairo, Egypt.

***Corresponding author e-mail:** aelmakawy@yahoo.com

**ABSTRACT**

Manjarix® is herbal drug composed of ginger and turmeric powdered as active compounds. The present study aimed to evaluate the potential *in vitro* and *in vivo* genotoxicity and developmental toxicity of manjarix. The genotoxicity assessment was conducted by *in vitro* Bacterial reverse mutation assay and *in vivo* chromosome aberrations and micronucleus test in rats. Bacterial reverse mutations assay was performed in Salmonella typhimurium strains and Escherichia coli (WP2-uvrA/) with and without metabolic activation system (S9 mix). For *in vivo* study, Manjarix in three doses (250,500 and 1000 mg /kg/daily) was tested. In the developmental toxicity study, Manjarix treated female and male rats were mated to evaluate the effect of Manjarix on their fetuses' development. Results revealed that Manjarix did not cause any genotoxic effects, as determined by the Ames test, *in vivo* chromosomal aberration and micronucleus assays. In addition, Manjarix did not show obvious alteration in the reproductive performance or fetal development. Based on these findings, we can conclude that the use of Manjarix in traditional medicine poses no risk.

**Keywords**: Manjarix, Genotoxicity, Bacterial reverse mutation, Chromosome aberrations, Micronucleated polychromatic erythrocytes, Developmental toxicity.

## INTRODUCTION

Herbal medicines have been extensively used in developed countries hence they are natural and relatively safe. [1] They contain plant materials as their pharmacologically active components. [2] According to the World Health Organization, about 80% of the world's population living in developing countries relies essentially on plants for primary health care. [3] Manjarix® is herbal drug composed of ginger powdered Extract from Rhizomes of Zingiber officinale Roscoe and Turmeric powdered Extract from Rhizomes of Curcuma longa L. as active compounds and used in women health care.

Ginger (Zingiber officinale Roscoe, Zingiberacae) is one of the most commonly used spices around the world, especially in the South-Eastern Asian countries. Ginger is also a medicinal plant that has been widely used in Chinese, Ayurvedic and Unani-Tibb medicines for a wide array of ailments that include arthritis, rheumatism, sprains, muscular aches, pains, sore throats, cramps, constipation, indigestion, vomiting, hypertension, dementia, fever, infectious diseases and helminthiasis. [4] Ginger represents a rich source of biologically active constituents. It is a strong antioxidant substance and may either mitigate or prevent the generation of reactive oxygen species. [5, 6] The active ingredients of Z. officinale roots and leaves including zingerone, Gingerdiol, zingibrene, Gingerols and shogaols have antioxidant activity. [7] In the fresh ginger rhizome, the gingerols were identified as the major active components and gingerol [5-hydroxy-1-(4-hydroxy-3-methoxy phenyl) decan-3-one] is the most

abundant constituent in the gingerol series. [8] Ginger has been demonstrated to have various pharmacological activities such as antiemetic, antiulcer, anti-inflammatory, antioxidant, anti-platelet, glucose- and lipid- lowering, cardiovascular, anti-cancer activities , anti-microbial activity , Neuro-protective activity and Hepatoprotective activities. [9-12] Ginger was reported to decrease age-related oxidative stress markers and was suggested to guard against hepatotoxicity by suppressing oxidative consequences. [13]

Curcumin, a phenolic compound extracted from the rhizomes of Curcuma longa, is worldwide used as spice, flavoring agent, food preservative, coloring agent, and as herbal medicine in Asian countries. Curcumin is nontoxic, even at high doses, and is classified by the FDA as "generally recognized as safe." A large body of evidence suggests that curcumin has a wide range of biological activities and potential therapeutic effects on numerous pathologic disorders, including diabetes, rheumatoid arthritis, multiple sclerosis, and cancer. [14-18] Clinical studies suggested that curcumin exhibits diverse and potent array of pharmacological effects in almost all of the major organ systems of the human body. These include anti-diabetic activity, anti-inflammatory activity, anticancer activity, antiaging, antifertility, hepactoprotective activity, anti HIV, opthamalic activity, antioxidant activity, antibacterial activity, antidepressant activity, cardiovascular and neurodegenerative diseases. [19]

Several controlled clinical trials have shown the efficacy of ginger in the treatment of nausea and vomiting during pregnancy. [20] A group that investigated the teratogenic potential of ginger extract found no embryotoxic or teratogenic effects. [21] Another group showed embryotoxicity associated with prenatal exposure of rats to ginger tea. [22] However, in a double blinded, randomized, crossover clinical trial, no teratogenic abnormalities were observed in infants born to mothers treated with ginger for severe vomiting during pregnancy. [23]
The present study was undertaken to evaluate the genotoxicity and the developmental toxicity of Manjarix. The genotoxicity study was conducted using bacterial reverse mutation assay and *in vivo* bone marrow chromosome aberrations and micronucleus assays. In addition, reproductive developmental study was conducted to evaluate the effect of parents' Manjarix administration on their fetuses.

## MATERIALS AND METHODS

**Plant materials and extracts preparation:** The extracts were obtained from TPM Biotech Sdn Bhd, Kuala Lumpur, Malaysia. Ginger Extract is the spray dried solvent extract of 100% water derived from the dried rhizomes of Zingiber officinale Roscoe, Fam. Zingiberaceae according to. [24] The plant parts were washed with distilled water, air-dried at room temperature then grinded into fine powder using mortar and pestle. 50 grams of powder was dissolved in 500 ml of distilled water and kept at room temperature for 24 hours for complete saturation. The saturated emulsion was filtered through a filter paper (Watman No.1). The filtrates were evaporated to dryness using thermostatically controlled water bath regulated to 100°C (spray dryer) and then stored in sterilized sample bottles. Turmeric Extract is the spray dried hydro-alcoholic extract of the dried rhizomes of Curcuma longa L., Fam. Zingiberaceae. Turmeric extract was by dissolving 50 g of dried powdered material in 500 ml of 30% ethanol at 60°C water bath for 1 h. The 30% ethanol liquid extract was filtered using Whatman No. 1 filter paper. The residue was re-extracted with another 500 ml of 30% ethanol. The two filtrates were combined and dried using rotatory evaporator at 60°C.

**Genotoxicity Assay**
**Bacterial Reverse Mutation Test**
***Positive control mutagens:*** Sodium azide (NaN3, Sigma), 9-aminoacridine, (9AA), 2-nitrofluorene (NF), benzo[a]Pyrene (BP), 2-aminoanthracene (2-AAN), Methyl methansulfonate (MMS) were purchased from Aldrich Chemical Company Limited.

***Bacterial strain:*** Salmonella typhimurium strains (TA 98, TA100, TA 97a, and TA 1535) and Escherichia coli (WP2-uvr A/) were purchased from Moltox (Molecular Toxicology Inc, USA). Metabolic activation system (S9 mixture) Lyophilized rat liver S9 fraction induced by Aroclor 1254 was purchased from Celsis, In vitro Technologies.

***Mutagenicity Assay:*** The Salmonella typhimurium assay was performed by the standard plate incorporation method with and without addition of a metabolic activation system (S9 mixture) according to OECD 471 (The OECD guideline for testing of chemicals in a Bacterial Reverse Mutation Test) under GLP complaint facility (TetraQ, Australia; GLP No. 15153). Basically, 100 µl of Manjarix aqueous extract at concentrations (100, 50, 10, 5, 2.5 and 1µg/plate) were added to 100µl of grown culture containing approximately $1–2\times10^9$ bacteria/ ml into

culture tubes which contained overlay agar. 50µl standard mutagens (positive control) or deionised water (negative control), and 500µl of sodium phosphate buffer (without S9) or 500µl of S9 mixture. After 72 hours of incubation at 37 °C, all plates were checked for the presence of the background lawn and compared to the negative control group plates.

***In Vivo Genotoxicity Assay***

**Animals:** Sprague-Dawley male and female rats weighing 120-150g were obtained from Misr University for Science and Technology, sixth of October, Egypt. The animals were acclimated for a period of one week before the beginning of the experiments. Rats were maintained under controlled of temperature (22±3°C), 50-55% relative humidity and light cycle of 12h light: 12h dark and were fed standard granulated diet and water ad labium. Animals were randomly divided into five groups of ten rats per group. The first group served as negative control and was administered orally distilled water at dose (10 ml/kg b. w.). The second group animals were injected IP with a single dose of 50mg/kg cyclophosphamide and used as positive control. The other three groups of rats were administered Manjarix orally at the dose levels of 250, 500 and 1000 mg/kg bw., once per day for 14 consecutive days. This study was conducted at Faculty of Pharmacy Animal Facility – Ain shams University in compliance with the OECD Good Laboratory Practice Principles and applicable Standard Operating Procedures.

***Bone Marrow Chromosome Preparation:*** At the end of the treatment, animals of all treated groups were injected intraperitoneally with colchicine to arrest cell division at metaphase. Two hours after injection, animals were sacrificed by cervical dislocation for chromosomes preparation of bone marrow cells using the methodology of Yosida & Amano. [25] Bone marrow cells were collected from both the femurs by flushing in saline solution. Bone marrow suspension was centrifuged at 1000 rpm for 10min and incubated at 37°C in hypotonic solution (KCl 0.56%) for 35min. Cells were fixed in methanol-glacial acetic acid (3:1). The cells were resuspended in a small volume of fixative, dropped onto chilled slides, flame-dried, and stained with 10% buffered Giemsa (pH 6.8).

***Bone Marrow Micronucleus Test:*** After 24 hrs of treatment, the animals were sacrificed and bone marrow was flushed out from the femur bone with fetal calf serum. The suspension of bone marrow cells was centrifuged for 10 min at 1000 rpm. The resulting sediment was resuspended in fetal calf serum smears were prepared from the resulting cell suspension. After air-drying and fixation in absolute methanol for10 min, slides were stained with Giemsa-stain. The slides were analyzed using a Nikon light microscope. For micronuclei (MNPCEs) evaluation, 2000 polychromatic erythrocytes were scored per animal. Both normochromatic erythrocytes (NCE) and polychromatic erythrocytes (PCE) were scored in 200 erythrocytes were counted for the PCE: NCE ratio according to the OECD guideline for testing of chemicals (mammalian erythrocyte micronucleus test), guideline No. 474 (OECD 1997).

***Reproductive Toxicity Study:*** Control and Manjarix three doses treated male and female rats were mated after the completion of the respective treatment regimen. The mating design was one male rat: two female rats. The day of sperm positive vaginal smear was considered as day zero of gestation. Gravid rats were sacrificed on day 21 of gestation and the number of implantation sites, corpora lutea, normal and resorbed fetuses were recorded. Potency, litter size, pre- and post-implantation loss were determined according to .

Potency: Potency is the ability of male rat to inseminate =

$$\frac{\text{Number of females inseminate}}{\text{Number of females exposed to mating}} \times 100$$

Litter size: Number of normal embryos/gravid female

Pre-implantation loss:

$$\frac{\text{No. of corpora lutea - No. of implantation sites}}{\text{No. of Corpora lutea}} \times 100$$

Post-implantation loss:

$$\frac{\text{No. of implantation sites - No. of live fetus}}{\text{No. of implantation sites}} \times 100$$

A batch of gravid females that mated with control and Manjarix treated male rats was allowed to deliver. All the pups were examined for gross morphological abnormality, their weight was noted and postnatal development was monitored.

**Statistical analysis**

Statistical analysis were performed using SPSS for Windows (Version 16). Data were compared by one-way analysis of variance (ANOVA) followed by Duncan's multiple range test. All values reported as means±SD. For all experimental data, the

significance level was set at P≤0.05, when appropriate.

## RESULTS

***Bacterial reverse mutation:*** The results of reverse mutation by Ames with and without S9 mix are summarized in Table (1). Data showed that there was no significant difference in the number of revertant colonies of any of the base pair substitution strains or frame shift mutation treated with Manjarix different doses with or without S9 mix relative to those of the corresponding negative control. However, the number of revertant colonies in positive control increased remarkably with or without S9 mix (p≤0.01).

***Bone marrow Chromosomal Aberrations Study:*** An *in vivo* chromosomal aberration study in both sexes of Sprague-Dawley rat was performed at three different doses of Manjarix and results are shown in tables 2 and 3. Analysis of chromosomal aberrations in bone-marrow cells showed that the treatment of the three doses of Manjarix induced a non-significant difference in the frequencies of individuals and total chromosomal aberrations compared with the negative control. Whereas, cyclophoshamide treated animals in both sexes has significantly increase (P≤0.01) in the mean values of different types of chromosomal aberrations.

***Bone Marrow Micronucleus Test:*** The frequency of micronucleated polychromatic erythrocytes (MNPCE) was calculated based on the observation of 2000 polychromatic erythrocytes (PCE) per animal to evaluate the genotoxic properties of Manjarix. Table 4 summarize the results of micronucleus test in bone marrow cells of Sprague-Dawley rats both sexes following treatment with different concentrations of the Manjarix and the controls. Results showed that the administration of Manjarix induced non-significant decrease in the frequencies of micronucleated polychromatic erythrocytes as compared to negative control. On the other hand, cyclophosphamide resulted in significant increase in the number of micronuclei in polychromatic erythrocytes (MNPCEs) compared with the control group.

**Developmental reproduction:** The reproductive performance of the control and Manjarix treated groups was represented in Table 5. Parents oral administration of Manjarix has not significantly changed the percentage of potency, litter size, pre- and post-implantation loss as compared to control. In addition, the results concerned with the number of total, live, dead embryos and litter weights at zero and 4th day of birth were summarized in Table 6. Data showed that there were no significant differences in the mean values of total numbers of embryos, live and dead embryo numbers at both observed ages. The mean body weight of fetuses sired with 250mg/kg Manjarix treated parents at day zero was significantly (p≤0.05) decreased as compared to control. While, there were no significant decrease in the mean body weights of fetuses sired with Manjarix 500 & 1000 mg/kg treated parents. In addition, there was no significant difference between body weights of parental Manjarix fetuses and control at the 4th day.

## DISCUSSIONS

Botanical products are generally intended as drugs, medicinal products, or substances for therapeutic use derived from raw material of whole plants or parts of them. These materials are processes through various stages such as extraction, distillation, purification, concentration, fermentation, and others. In many countries these products are regulated both as medicinal products and they are often labeled as natural supplements. [26] Several studies have demonstrated that natural products can represent serious risks to the DNA integrity of different organisms. [27, 28]

Such information underlines the importance of studying the genetic risks of plant compounds, especially that utilized by humans in medicinal treatments. In the present study, we evaluated the potential genotoxicity of Manjarix using the bacterial reverse mutation assay, *in vivo* chromosomal aberrations and micronucleus assays in Sprague-Dawley rat bone marrow cells. The Ames bacterial test done with mutagenic S. Typhimurium, has been used with good success to screen mutagenic properties of different agents. [29] The Ames assay has been validated as a mutagenicity assay in different laboratories and yield results comparable to *in vivo effects*. [30]

Results of Ames assay showed that Manjarix tested doses did not induced any significant increase in the number of relevant colonies than the negative control, either in the presence or in the absence of extrinsic metabolic activation. This result was in agreement with previous studies conducted by using some compound that isolated from Zingiber zerumbet. Al-Zubairi [31] reported that Zerumbone a natural compound isolated from ginger failed to induce mutagenic effects on Salmonilla Typhimurium strain TA100 in the presence or

absence of S9 liver metabolic activation system. Chang et al. [32] results indicate that alcoholic extracts of Zingiber zerumbet (L.) does not induce mutagenicity in several strains of Salmonella typhimurium, as determined by the Ames test. Meanwhile, the results of the *in vivo* genotoxicity assessments demonstrated clearly that there were no significant increases in the frequencies of chromosomal aberrations or MNPCEs at any dose of Manjarix. These findings indicating that Manjarix is not have mutagenic or genotoxic activity and were in agreement with Bidinotto et al. [33] they confirmed that ginger not genotoxic. Chang et al. [32] indicated that Zingiber zerumbet caused no significant increases in the number of micronucleated polychromatic erythrocytes (MNPCEs) and mean ratio of polychromatic erythrocytes to total erythrocytes.

In addition, Kota et al. [34] reported that turmeric did not show any significant effect on the mutagenic index and may be considered as non-mutagenic. Thus, the results of the *in vivo* assay corroborate those of the *in vitro* mutagenicity test. Both assays strongly suggest that the consumption of Manjarix does not pose any genotoxic hazards.

As regards to the developmental toxicity study, results illustrated that parents oral administration of Manjarix has not significantly changed the percentage of potency, litter size, pre- and post-implantation loss as compared to control. In addition, there were no significant differences in the total numbers of embryos, live and dead embryo numbers at both observed ages. Preliminary studies suggest that ginger may be safe and effective for nausea and vomiting of pregnancy when used at recommended doses for short periods. Boone and Shields [23] found that different doses of ginger were safe and effective to treat pregnancy sickness during the period of pregnancy.

Some safety concerns have been raised when pregnant women used ginger in large doses. [35] In a study by Dissabandara & Chandrasekara [36] pregnant rats were administered dried powder extract of ginger orally at doses of 500 or 1000 mg/kg daily during gestation days 5 to 15. Duration of pregnancy, litter size, number of implantation sites and live birth index were not altered by ginger, however a statistically significant higher number of embryo resorption was observed in both test groups.

Betz et al. [37] confirmed that a daily dose of 6 g of ginger could be used for pregnancy sickness treatment with few side effects as intestinal symptoms, sleeping and one abortion case from 136 women at the 12$^{th}$ week of the pregnancy. In addition, Ganiger et al. [38] showed that rats fed diet containing 0.5% turmeric for 12 weeks did not have any adverse effect on pregnancy rate, mean number of live and dead embryos. Data of this work was confirmed with the study of Ali et al. [4] they mentioned that ginger is a strong antioxidant substance and may either mitigate or prevent generation of free radicals. It is considered a safe herbal medicine with only few and insignificant adverse/side effects.

## CONCLUSION

Based on the findings of the present study, we can conclude that Manjarix aqueous extract was not inducing genotoxicity or developmental toxicity. Therefore can be used for applications of traditional medicine in modern complementary and alternative therapeutics and health care.

## ACKNOWLEDGMENT

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**Table 1: Mutagenicity assay for Manjarix with and without-metabolic activation using *S. typhimurium* and *E. coli* strains.**

| Concentration of test material (µg/plate) | | Average of revertant colonies (Mean ±SD) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Base Pair Substitution | | | | | | Frame Shift mutation | | | |
| | | TA 100 | | TA 1535 | | WP2 uvrA | | TA 98 | | TA 97a | |
| | | -S9 | +S9 | -S9 | +S9 | -S9 | +S9 | -S9 | +S9 | -S9 | +S9 |
| **Manjarix** | 1.0 | 0.57±0.01 e | 0.67±0.01 e | 0.51 ±0.01 d | 0.61±0.01 d | 0 .0±0.0 c | 0 .0±0.0 e | 0.16±0.02 d | 0.26±0.01 d | 0.12±0.02 d | 0.22±0.02 d |
| | 2.5 | 1.96±0.02 d | 2.92±0.06 d | 1.16±0.02 d | 1.26±0.02 d | 0.54±0.01 c | 0.64±0.01 e | 0.52±0.02 d | 0.62±0.03 d | 0.16±0.02 d | 0.26±0.02 d |
| | 5.0 | 2.37±0.02 d | 3.16±0.27 d | 6.13±0.15c | 7.42±0.43 c | 2.78±0.02 c | 2.87±0.02 d | 0.90±0.03 d | 1.24±0.23 d | 0.32±0.02 d | 0.47±0.11 d |
| | 10 | 6.02±0.06 c | 7.02±0.11 c | 16.00±0.0 b | 17.35±0.4 b | 8.67±0.0 b | 9.98±0.28 c | 3.20±0.04 c | 3.80±0.26 c | 0.88±0.02 d | 0.97±0.25 d |
| | 50 | 6.04±0.08 c | 7.37±0.41 c | 16.01±0.0b | 17.68±0.4 b | 8.67±0.0 b | 9.70±0.52 c | 3.20±0.04 c | 3.98±0.17 c | 3.83±0.03 c | 4.73±0.25 c |
| | 100 | 6.02±0.06 c | 7.38±0.48 c | 16.00±0.0 b | 17.55±0.2 b | 8.67±0.0 b | 9.80±0.18 c | 3.20±0.04 c | 4.25±0.25 c | 3.83±0.03 c | 4.70±0.43 c |
| Historical negative (background) | | 16.00±1.00 b | | 7.00±1.00 c b | | 12.00±1.00 | | 31.66± 1.53 b | | 26.00±1.00 b | |
| Positive control | | NaN3 | | NaN3 | | ENU | | 2NF | | 9AA | |
| | | 88.00±1.00 a | | 24.66±4.37 a | | 93.02±6.12 a | | 71.67±1.53 a | | 83.00±1.73 a | |

Mean values followed with different letters within the same column are significantly different from one another (P≤0.05).

**Table2: Chromosome aberrations induced in bone marrow cells of male rats treated with Manjarix® for 14 days**

| Items | | -ve control | ±ve control | M 250mg/kg | M 500mg/kg | M 1000mg/kg |
|---|---|---|---|---|---|---|
| Numerical chromosomal aberrations | n- | 2.40± 1.14 b | 3.60 ±0.54 a | 1.20±0.44 c | 1.40 ±0.54 c | 1.60±0.54 bc |
| | n± | 0.00 ±0.00 b | 1.60±0.54 a | 0.40 ±0.54 b | 0.40±0.54 b | 0.60 ±0.54 b |
| | Polyploidy | 1.00±0.01 b | 2.40 ±0.89 a | 1.25 ±0.50 b | 1.20 ±0.83 b | 1.50±0.57 b |
| | Total | 3.40±1.14 b | 7.60 ±1.14 a | 2.60±0.54 b | 2.60±1.14 b | 3.40±0.54 b |
| Structural chromosomal aberrations | Dicentric | 0.60±0.54 b | 2.00 ±1.22 a | 0.80 ±0.44 b | 0.60±0.54 b | 1.00±0.01 ab |
| | CF | 0.00 ±0.00 b | 2.00 ±0.70 a | 0.60±0.54 b | 0.60±0.54 b | 0.00 ±0.00 b |
| | Ring | 0.00 ±0.00 c | 1.80 ±0.44 a | 0.40±0.54 bc | 0.60±0.54 bc | 1.00 ±0.01 b |
| | Break | 2.00 ±0.70 b | 4.40±1.67 a | 0.80±0.44 b | 1.00±0.01 b | 0.80 ±0.83 b |
| | Chr gap | 0.00 ±0.00 b | 0.60±0.54 a | 0.00 ±0.00 b | 0.00 ±0.00 b | 0.00 ±0.00 b |
| | Cht gap | 2.00 ±0.71 b | 3.20 ±0.83 a | 1.80±0.44 b | 1.20±0.83 b | 1.20±1.09 b |
| | Del | 0.00 ±0.00 c | 3.00±1.14 a | 0.80±0.44 bc | 0.80±0.83 bc | 1.50 ±0.57 b |
| | End to end | 0.00 ±0.00 c | 2.20±0.83 a | 1.00±1.00 ab | 1.20±1.30 a | 1.40±0.54 a |
| | CA | 3.00±0.70 b | 6.00±0.70 a | 2.80±10.44 b | 3.00± 1.22 b | 2.50±0.57 b |
| | Fragment | 1.60±0.50 b | 3.40±1.81 a | 1.00±0.70 b | 0.60±0.54 b | 1.33±0.57 b |
| | Total | 9.20±1.09 b | 28.80±1.78 a | 10.00±1.34 b | 9.60±1.14 b | 8.60±1.34 b |
| Total chromosomal aberrations excluding gap | | 10.00±1.22 b | 32.60 ±3.50 a | 10.80 ±0.83 b | 11.00±0.70 b | 11.80±1.48 b |
| Total chromosomal aberrations including gap | | 12.60 ±0.54 b | 36.20 ±2.16 a | 12.80 ±1.09 b | 12.40 ±1.67 b | 12.00 ±1.00 b |

Data are presented as Mean ± S.D. Statistical analysis was carried out by one way ANOVA followed by Duncan's post hoc Test.
Mean values followed with different letters within the same column are significantly different from one another (P≤0.05).

**Table 3: Chromosome aberrations induced in bone marrow cells of female rats treated with Manjarix® for 14 days**

| Items | | -ve Control | ±ve Control | M 250 mg/kg | M 500 mg/kg | M 1000 mg/kg |
|---|---|---|---|---|---|---|
| Numerical chromosomal aberrations | n- | 2.00 ±0.70 a | 2.20 ±0.83 a | 1.60±0.54 a | 1.40 ±0.54a | 1.60 ±0.54 a |
| | n± | 0.00 ±0.00 b | 1.50 ±0.57a | 0.20±0.44 b | 0.40±0.54 b | 0.40±0.54 b |
| | Polyploidy | 1.00 ±0.01 b | 2.20±0.83 a | 1.00±0.70 b | 1.00±0.03 b | 1.00±0.02 b |
| | Total | 2.60±0.89 b | 5.60±0.54 a | 2.80±0.44 b | 2.60±0.54b | 2.60±0.54 b |
| Structural chromosomal aberrations | Dicentric | 0.80±0.44 b | 2.00 ±0.70 a | 0.60±0.54 b | 0.80±0.44 b | 0.80±0.44 b |
| | CF | 0.00 ±0.00 b | 2.75±0.50 a | 0.20±0.44 b | 0.40±0.54 b | 0.40±0.54 b |
| | Ring | 0.00 ±0.00 b | 1.40±0.54 a | 0.00 ±0.00 b | 0.00 ±0.00 b | 0.20±0.44 b |
| | Break | 1.80±0.44 b | 3.60±1.94 a | 1.20±0.44 b | 1.25±0.95 b | 1.25±0.50 b |
| | Chr gap | 0.00 ±0.00 b | 0.60±0.54 a | 0.00 ±0.00 b | 0.00 ±0.00 b | 0.00 ±0.00 b |
| | Cht gap | 2.00±0.05 b | 3.40±0.54 a | 1.50±0.57 b | 1.20±0.83 b | 1.40±0.54 b |
| | Del | 0.00 ±0.00 c | 2.50 ±0.57 a | 0.40±0.54 bc | 1.00 ±0.81 b | 1.00±0.01 b |
| | End to end | 0.00 ±0.00 c | 2.60±0.89 a | 0.40±0.54 bc | 0.80±0.44 b | 1.00 ±0.04 b |
| | CA | 2.60±0.54 b | 5.20±0.83 a | 2.60 ±0.54 b | 2.00±0.70 b | 1.60±0.89 b |
| | Fragment | 1.00±0.54 c | 2.20±0.83 a | 1.40±0.54 ab | 1.50±0.57 ab | 1.25±0.50 c |
| | Total | 8.00±1.00 b | 25.00±2.73 a | 8.00±1.58 b | 8.00±1.00 b | 7.80±0.83 b |
| Total chromosomal aberrations excluding gap | | 8.80±0.83 b | 27.60±3.28 a | 9.60±1.14 b | 9.40±0.89 b | 9.00±0.70 b |
| Total chromosomal aberrations including gap | | 10.80±0.83 b | 30.60±2.88 a | 10.80±1.30 b | 10.60±0.54 b | 10.40±0.54 b |

Data are presented as Mean ± S.D. Statistical analysis was carried out by one-way ANOVA followed by Duncan's post hoc Test.
Mean values followed with different letters within the same column are significantly different from one another (P≤0.05)

**Table 4: Frequencies of micronucleated polychromatic erythrocytes in bone marrow cells of rats treated with Manjarix for 14 days**

| Treatment | Dose mg/kg b.w. | Frequency of MNPCEs M ± SD | | MNPCEs % M ± SD | | PCE/NCE ratio | |
|---|---|---|---|---|---|---|---|
| | | ♂ | ♀ | ♂ | ♀ | ♂ | ♀ |
| - ve Control | 0 | 8.20±1.48 b | 8.80±0.83 b | 0.41±0.07 b | 0.44±0.04 b | 7.79 ± 0.10 b | 7.41±0.09 b |
| | 50 | 38.20 ±9.12 a | 35.20 ±9.20 a | 1.91±0.45 a | 1.76±0.46 a | 4.88± 0.13 d | 4.71±0.11 d |
| ± ve Control | 250 | 8.20 ±0.83 b | 8.60 ±1.14 b | 0.41±0.04 b | 0.43±0.05 b | 7.02 ±0.20 c | 6.86±0.12 c |
| | 500 | 7.60 ±0.54 b | 8.40 ±1.14 b | 0.38±0.02 b | 0.42±0.05 b | 8. 2 6 ±0.18 a | 7.85±0.16 a |
| Manjarix | 1000 | 7.40 ±0.54 b | 8.20 ±0.83 b | 0.37±0.02 b | 0.41±0.04 b | 8.44 ±0.42 a | 8.02±0.38 a |

Two thousand cells were analyzed per animal, for 10000 cells per group. Data were expressed as Mean ± SD
Mean values followed with different letters within the same column are significantly different from one another (P≤0.05).

**Table 5 : Reproductive performance of control and Manjarix treated rats**

| Treatments | % Potency | Pre-implantation loss | Litter size | Post-implantation loss |
|---|---|---|---|---|
| Control | 80 | 2.13±0.27 a | 7.80±0.42 a | 0.73±0.74 a |
| Manjarix 250mg/kg | 60 | 1.13±0.32 b | 6.80±0.31 a | 0.53±0.29 a |
| Manjarix 500mg/kg | 70 | 1.20±0.26 b | 6.87±0.35 a | 0.47±0.16 a |
| Manjarix 1000mg/kg | 80 | 1.67±0.28 ab | 7.00±0.48 a | 0.87±0.36 a |

Data were expressed as Mean ± SD Mean values followed with different letters within the same column are significantly different from one another (P≤0.05).

**Table 6 : Reproductive findings of pregnant control and Manjarix treated rats**

| Treatment | No. of embryos | No. of female embryos | No. of male embryos | No. of dead embryos at day | | No. of live embryos at day | | Litter weights at day | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | **0** | **4** | 0 | 4 | 0 | 4 |
| Control | 7.80±0.42 a | 3.33±0.39 ab | 4.47±0.36 a | 0. 07±0.06 a | 0.07±0.06 a | 7.73±0.43 a | 7.73±0.43 a | 45.60±2.68 a | 71.19±4.32 a |
| Manjarix 250mg/kg | 6.80±0.31 a | 3.80±0.27 a | 2.93±0.38 b | 0.07±0.06 a | 0.53±0.35 a | 6.67±0.31 a | 5.80±0.67 b | 37.30±1.92 b | 60.63±2.97 a |
| Manjarix 500mg/kg | 6.87±0.35 a | 2.73±0.24 b | 4.13±0.35 a | 0 a | 0.40±0.40 a | 6.87±0.35 a | 6.47±0.57 ab | 42.19±2.27 ab | 67.61±3.34 a |
| Manjarix 1000mg/kg | 7.00±0.48 a | 3.20±0.39 ab | 3.80±0.38 ab | 0 a | 0.07±0.06 a | 7.00±0.48 a | 6.93±0.48 ab | 42.60±3.46 ab | 67.26±4.48 a |

Data were expressed as Mean ± SD Mean values followed with different letters within the same column are significantly different from one another (P≤0.05).

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