

Effect of Mirazid on the Micronuclei Polychromatic Erythrocyte Cells (MNPCEs) In the Bone Marrow Cells of *Schistosoma* Infected and Non Infected Mice

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Abstract—In the present investigation, the efficacy of *Commiphora molmol* (Mirazid[®]) in treating schistosomiasis was cytogenetically studied by bone marrow cells.

The percentage frequency of micronuclei polychromatic erythrocyte cells (MNPCEs) in bone marrow cells of mice infected mice with *Schistosoma* was higher than the other groups. The MNPCEs of non-infected treated and recovery groups was higher than control. The percentage of MNPCEs of infected treated recovery group was similar to that of the infected treated group. The bone marrow activity reached a maximum value in the infected group and decreased in the other groups.

Keywords— Mirazid[®], schistosomiasis, micronucleus test. Mice.

I. INTRODUCTION

Mirazid (myrrh) is an antischistosomal drug which is an extract of an oleo-gum resin obtained from the stem of the plant *Commiphora molmol* (1,2). *Commiphora* species has been exploited as a natural drug to treat pain, skin infections, inflammatory conditions, diarrhea and periodontal diseases. Products derived from various species of *Commiphora* are recognized to possess significant antiseptic, anesthetic and antitumor properties (3, 4). Additionally, *C. molmol* showed significant antimutagenic, antioxidative and cytoprotective properties (5, 6, 7).

Weitzman and Gordon (8) recorded that the reactive oxygen produced by inflamed leucocytes and macrophages during *Schistosoma* infection induce DNA strand breakage, chromosomal damage and malignant transformation in mammalian cells. The classic cytogenetic analysis of chromosomal aberrations in metaphases is the gold standard of biological dosimeter for many years, but the complexity of this technique and the long time and efforts required, lead to the development of another technique known as the micronucleus test. This test is used in the present investigation because it is simple, fast and does not need large number of cells to be screened. Micronuclei are small bodies in the cytoplasm resembling the nuclear material in morphology and staining pattern. They are formed when a broken chromosome or chromosome fragment does not travel to the spindle fibers during mitosis and therefore are not included in daughter

nuclei but appear in daughter cytoplasm. Furthermore, the bone marrow cells of mice were used because of their high mitotic rate and they can be obtained without culturing in the metaphase stage. Also, erythrocytes are particularly well suited for evaluating micronucleus events, since erythroblast precursors are rapidly dividing population of cells and their nucleus is expelled a few hours after the last mitosis, making micronucleus associated chromatin relatively simple to detect. So, the effect of Mirazid on micronucleus can be tested easily. The present study aimed to evaluate the efficiency of *Commiphora molmol* (Mirazid) in the treatment of schistosomiasis through micronucleus test.

II. MATERIALS AND METHODS

The cytogenetic study was carried out through micronucleus test in bone marrow cells. A total of Thirty non-infected mature male mice, weighing about 18 ± 2 gm, were obtained from the experimental animals' farm, Faculty of Veterinary Medicine, Zagazig University, Egypt. The animals were accommodated to the laboratory conditions for at least two weeks before being used. The cercariae infected mice (age 8 weeks and weight 18 ± 2 gm) were purchased from Theodor Bilharzia Research Institute (Cairo). They were kept in a controlled environment for 8 weeks before experiments.

The active compounds in the mirazid are resins (including a-, b- & g-Commiphoric acids, Commiphorinic acid, heeraboresene, a-and b-heerabomyrrhols and commiferin) and the safety dose for remedy as anthelmintic is infusion. To make an infusion the resin should be powdered well as it dissolves in water with difficulty. Then, pour a cup of boiling water onto 1-2 tea spoonful's of resin powder and leave it to infuse for 10-15 minutes. This should be drunk three times a day.

Micronucleus test: Thirty mature male mice were used to study the effect of mirazid on the appearance of micronucleus polychromatic erythrocytes. There were classified as: GI: 5 mice were kept as a control group. GII: 10 mice were injected orally with 0.25 ml of mirazid for 6 successive days. Five mice were sacrificed and the micronucleus test was applied using bone marrow cells and the other five were kept as a recovery group for 10 days, then animals were sacrificed for the micronucleus test. GIII: 5 infected mice with cercariae were kept 8 weeks, and then sacrificed and the micronucleus test was applied. GIV: 10 mice infected with cercariae were kept for 8 weeks. Five mice were sacrificed and micronucleus test was

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applied using bone marrow cells post treatment with mirazid after 6-successive days. The remained 5 mice were kept as a recovery group for 10 days then sacrificed and micronucleus test was applied using bone marrow cells.

Micronucleus assay: After animals were sacrificed, the femur bone marrow was flushed in 6ml fetal calf serum, the suspension was centrifuged at 1000 rpm for 10 minutes. The supernatant was discarded except for 1ml and the pellet of cells were flushed and then spread on clean glass slides and fixed by methanol for 15 minutes, stained with 1.5% Giemsa for 10-15 min. Slides were coded and 1000 polychromatic erythrocytes were examined X1000 magnification. Micronuclei polychromatic erythrocytes were recorded and photomicrographs were taken. Statistical analysis was evaluated by a dispersion test (9).

III. RESULTS

The results are shown in tables (1) and figs. (1-4).

TABLE 1: COMPARISON BETWEEN FREQUENCIES OF MICRONUCLEI POLYCHROMATIC ERYTHROCYTE CELLS (MNPCEs) FROM BONE MARROW CELLS FOR INFECTED AND NON-INFECTED MICE TREATED WITH MIRAZID

Treatment group	No. of mice	No. of cells 1000/ mouse	Micronuclei polychromatic erythrocyte cells			bone marrow activity (mean/1000)
			No	%	Mean \pm S.E	
Control g	5	5000	94	1.88	18.8 \pm 1.46	0.0188
non- Infected	5	5000	179	3.58	35.8 \pm 4.75*	0.0358
non-Infected recovery	5	5000	131	2.62	26.2 \pm 4.84	0.0262
Infected	5	5000	382	7.64	76.4 \pm 7.22***	0.0764
Infected treated	5	5000	246	4.92	49.2 \pm 7.16*	0.0492
Infected treated recovery	5	5000	248	4.96	49.6 \pm 9.57*	0.0496

* Significant at $P < 0.05$, ** Significant at $P < 0.01$, *** Significant at $P < 0.001$

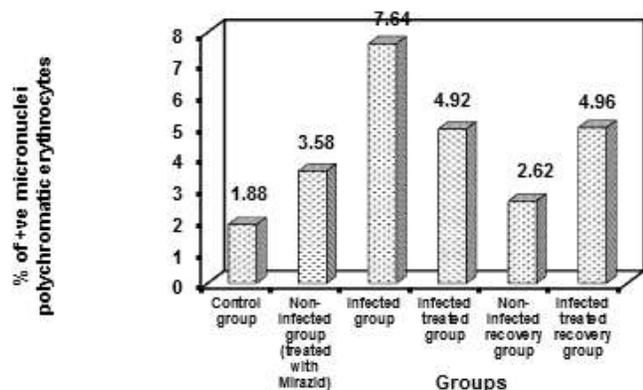


Fig. 1: Comparison between micronuclei polychromatic erythrocyte% from bone marrow cells of non infected and infected mice with cercariae and then treated by mirazid emulsion

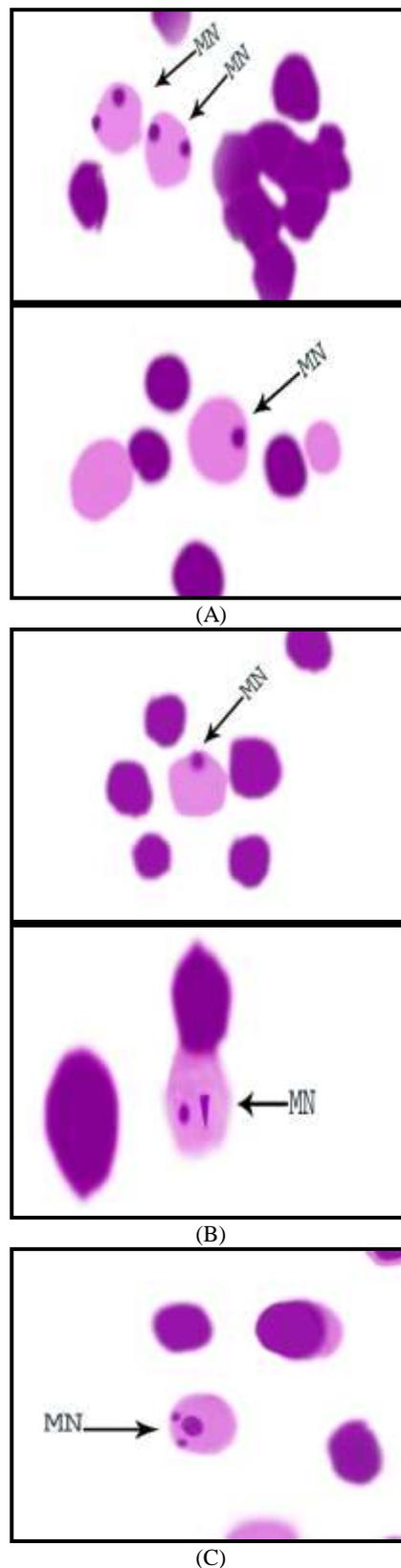


Fig. (2): Micronucleated polychromatic erythrocyte prepared from bone marrow cells of non-infected male mice treated with mirazid (A) Single MN (Round - shape) (B) Double MN (Round and Rod- like structure) (C) triple. MN (small & large)

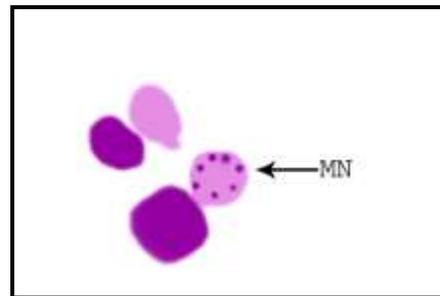
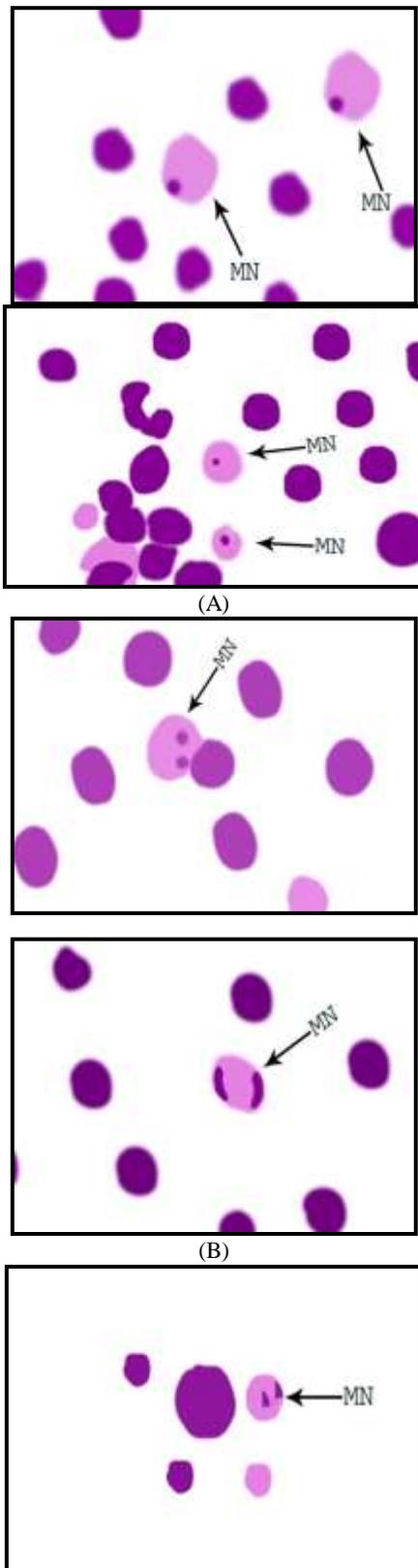
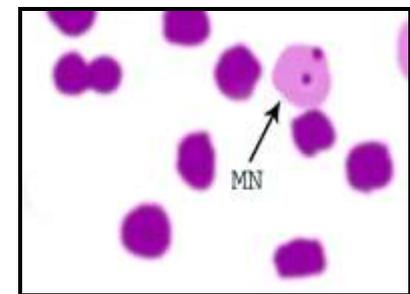
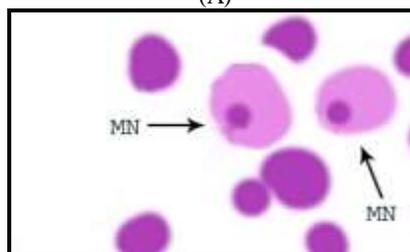
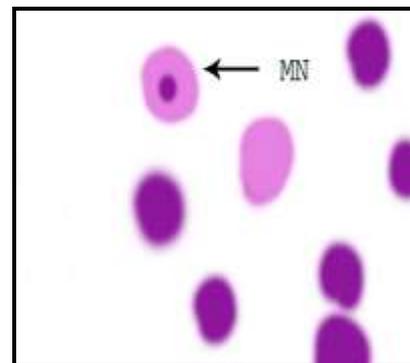
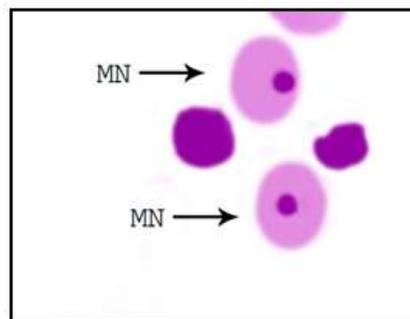
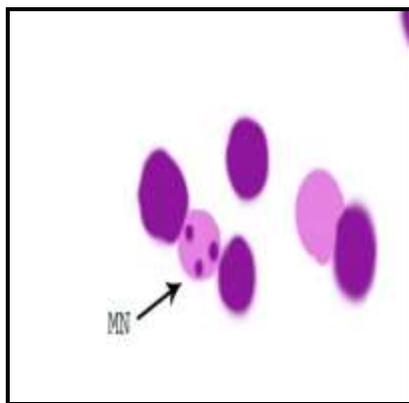


Fig. (3): Micronucleated polychromatic erythrocyte prepared from bone marrow cells of infected male mice with cercariae.

(A) Single MN (Dot-like structure and round shape)
 (B) Double MN (round, rod-like structure and almond shape). (C) Multi MN.





(C)

Fig. (4): Micronucleated polychromatic erythrocyte prepared from bone marrow cells of infected mice and treated with mirazid. (A) Single MN (round – shape) (B) double MN (Dot-like structure and round shape) (C) triple MN

In comparison between MNPECs from non-infected mice group and infected one, the MNPECs% in bone marrow cells of infected mice was progressively higher than other groups. However, the MNPECs% of non-infected recovery group was reduced, but still higher than control group (Fig. 1).

IV. DISCUSSION

In the present work, the Mirazid drug is used as an antischistosomal drug which possess good oxygen radical scavenger that reduced malondialdehyde concentration in carcinoma cells after using it in the treatment

In the present work, the frequency of micronucleated polychromatic erythrocyte cells from bone marrow of male mice infected with cercaria and the percentage of bone marrow activity for the formation of micronuclei were high. Moreover, the group of mice treated with only mirazid was less affected and the infected group which was treated with Mirazid was reduced. Blood cells are the most sensitive tissue to any infection, whenever, the MNPECs in bone marrow cells are complementary to the chromosomal aberration in leucocytes of bone marrow cells. In the same time the harvested micronuclei varied in size (small or large), shape (dot, round, almond) and number (single, double or more).

These data are complementary to the types of damage in chromosomes. The large and almond micronuclei represented whole chromosome lost during anaphase stage or during degeneration of erythrocyte nucleus. Moreover, the small and multi-micronuclei cells represented acentric fragment. These data agreed with (10) who reported that schistosomiasis is involved in the incidence of several cancers. Urothelial cells collected from patients infected with *S. haematobium* were shown to have an increased frequency of micronuclei formation suggesting the induction of chromosome injury. And (11) found that *Schistosoma* infection elevated the sister chromatid exchange and chromosomal aberration in blood lymphocytes, *C. molmol* significantly reduced the polychromatic erythrocyte/Normochromatic erythrocyte ratio indicating its cytotoxic potential.

In contrast, (12) reported that schistosomiasis did not have clastogenic effect but considered as a comutagen. However, it

was devoid of any clastogenic activity in the femoral cells of normal mice (13). In addition, the levels of DNA & proteins in hepatic cells were not affected by *C. molmol* treatment in normal mice (14).

In addition the myrrh proved to be a safe effective drug and the most frequently reported side effects were giddiness, somnolence and mild fatigue (15).

In this investigation, the recovery that occurred within 10 days denoted that the mirazid drug is not only a therapy but also help in the recovery of the side effects induced in the the micronuclei polychromatic erythrocytes from the *Schistosoma* infection. The present data agreed with (16) who reported that myrrh is a well tolerated remedy with high margin of safety for the liver, kidney, haemopoietic system and chromosomes.

V. CONCLUSION

The present results proved that the antischistosomal drug (Mirazid) extracted from myrrh caused protection against clastogenic effect of schistosomiasis on the micronuclei polychromatic erythrocytes. In view of the outcome data and the contradictory reports in the literature, it is important to carry out more studies using various types of cells under different experimental conditions to resolve the controversy concerning the possible risks associated with the extraction of phytoconstituents.

REFERENCES

- [1] Hall, B.L., Oserb, B.L. (1965): Progress in the consideration of flavoring ingredients under the food additive amendment: 3- GRAF substances. Food Technol. 19:151-97
- [2] Ford, R.A., Api, A.M., Letizia, C.S. (1992): Monographs on fragrance to raw materials. Food Chem. Toxicol. 30:91-2.
- [3] Nomicos, E. (2007): Myrrh: Medical larval or Myth of the Magi. Holist. Nurs. Pract. 21, 6:308-23. <https://doi.org/10.1097/01.HNP.0000298616.32846.34>
- [4] Paraskeva, M.P., Van Vuuren, S. F., Vanzyl, R.L., Davids, H., Vilj-oen, A. M. (2008): The in vitro biological activity of selected South African Commiphora species. J. Ethnopharmacol. 119, 3:673-9. <https://doi.org/10.1016/j.jep.2008.06.029>
- [5] Qureshi, S., Al-Harbi, M.M., Ah-med, M.M., Raza, M., Giangreco, A.B., Shah, A.H. (1993): Evaluation of the genotoxic, cytotoxic, and antitumor properties of Commiphora molmol using normal and ehrlich ascites carcinoma cell-bearing Swiss albino mice. Can. Chemother. Phar- macol. 33:130-8.
- [6] Al-Harbi, M.M., Qureshi, S., Raza, M., Ahmed, M.M., Giangreco, A.B., Shah, A.H. (1994): Anticarcinogenic effect of Commiphora mol-mol on tumors induced by Ehrlich carcinoma cells in mice. Exp. Chemother. 40:337-47. <https://doi.org/10.1159/000239216>
- [7] Al-Harbi, M.M., Qureshi, S., Raza, M., Ahmed, M.M., Afzal, M., Shah, A.H. (1997): Gastric antiulcer and cytoprotective effect of Commiphora molmol in rats. J. Ethnophar- macol. 55, 2:141-50. [https://doi.org/10.1016/S0378-8741\(96\)01488-2](https://doi.org/10.1016/S0378-8741(96)01488-2)
- [8] Weitzman, S.A., Gordon, L.I. (1990): Inflammation and cancer, role of phagocyte-generated oxidants in carcinogenesis. Blood 76:655-63.
- [9] Snedecor, G.W.; Cochran, W.G. (1976): Statistical Methods. 6th edition, Iowa State University Press, Ames, Iowa.
- [10] El-Sebai, M. (1978): Cancer of bilharzial bladder. Urol. Res., 6:233-6. <https://doi.org/10.1007/BF00262625>
- [11] Shubber, E.K., Salih, H., 1987: Cytogenetic studies on blood lymphocytes from patients with Schistosoma mansoni. J. Med. Sci. Biol. 40, 4:137-45 <https://doi.org/10.7883/yoken1952.40.137>
- [12] El-Sharkawy, I.M., Saleh, W.A., El-Alfy, N.M. (2003): Cytogenetics of acute and chronic schistosomiasis mansoni. J. Egypt. Soc. Parasitol. 33, 2: 341-52.

- [13] Raafat, M., El-Gerzawi, S., Stich, H.F. (1984): Detection of mutagenicity in urothelial cells of bilharzial patients, micronucleus test. *J. Egypt. Nat. Cancer Inst.* 1:63-73.
- [14] Qureshi, S., Shah, A.H., Tariq, M., Ageel, A.M. (1989): Studies in herbal aphrodisiacs used in Arab system of medicine. *Amer. J. Chinese Med.* 16:57-62.
<https://doi.org/10.1142/S0192415X89000103>
- [15] Sheir, Z., Amira, A., Massoud, A., Salama, O., Gamal, A., et al. (2001): A safe, effective, herbal, antischistosomal therapy derived from myrrh. *Am. J. Trop. Med. Hyg.* 65, 6:700-4.
- [16] Omar, A.M., El-Mesallamy, G.E., Eassa, S. (2005): Comparative study of the hepatotoxic, genotoxic and carcinogenic effects of Praziquantel (Distocide) and the natural myrrh extract (Mirazid) on adult male albino rats. *J. Egypt. Soc. Parasitol.* 35, 1:313-29.