Dept. of Microbiology, Fac. of Medicine,
Head of prof. Dr. M.S. El-Reheewy.

IMMUNOLOGICAL AND HEMATOLOGICAL CHANGES AFTER PRAZIQUANTEL TREATMENT IN DEN-INDUCED HEPATIC NEOPLASIA IN RATS

(With one Table and 3 Fig)

By

M.T. SHATA , M. TAHA* and A. SH. SEDDEK**

(Received at 13/1/1993)

التغيرات المناعية وصورة خلايا الدم بعد معالجة الأورام الكبديه بالبرازى كوانتيل المستحدثه بالداى ايثيل نيتروزامين فى الفئران

طارق شطا ، مختار طه ، عبد اللطيف شاكر

فى هذا البحث تم دراسة التأثير المناعى وصورة خلايا الدم للبرازى كوانتيل على الفئران الحامله لسرطانات الكبد المستحدثه بالداى ايثيل نيتروزامين الذى تم اطائه لعدد ٥٠ فأر عن طريق ماء الشرب يوميا ولمدة ١٢ أسبوع وقد أستعمل ٢٠ فأر كمجموعة ضابطة وعند ظهور التغيرات الكبديه ما قبل السرطانية (عند الاسبوع السادس من الحقن) قسمت الفئران المحقونة الى مجموعتين. المجموعه الاولى تكونت من ٢٠ فأر وتم معالجتها بالبرازى كوانتيل بجرعة ٣٠ مجم/ كمجم يوميا فى ماء الشرب لمدة ٦ ايام متتالية بالاضافه الى استمرارها فى تعاطى المادة المحدثه بالسرطان بنفس الكيفية. أما المجموعة الثانية فقد تكونت من ٢٠ فأر واستمرت فى تعاطى الداى ايثيل نيتروزامين حتى نهاية التجربة. وقد تم تجميع وفحص عينات من دم ومصل الفئران فى كل من المجموعتين بالاضافة الى المجموعة الضابطة قبل بداية التجربة وعند الاسبوع الثانى عشر من الحقن. وقد زادة نسبة الجلوبيولبنات المناعية الكلية ثلاث أضعاف فى فئران المجموعة الاولى بالمقارنة بالمجموعة الضابطة وانعكست هذه الزياده فى فئران المجموعة المعالجة بالبرازى كوانتيل. كما وجد أن تعاطى البرازى كوانتيل فقط ليس له تأثير على الفئران بالنسبة للجلوبيولينات المناعية الكلية وقد زاد العدد الكلى لخلايا الدم الحمراء وحجم الخلايا المضغوطه بعد تعاطى الداى أيثيل نيتروزامين وأنعكست هذه الزيادة بعد المعالجة بالبرازى كوانتيل. كما وجد أن البرازى كوانتيل فقط ليس له تأثير على هذه الخلايا . وقد أدى تعاطى الداى ايثيل نيتروزامين الى زيادة نسبة هيموجلوبين الدم فى فئران المجموعة الاولى ولم تنخفض هذه الزياده فى المجموعة بالبرازى كوانتيل. ولم تكن هناك أى زيادة معنوية فى عدد خلايا الدم البيضاء أو تصنيفها بالمقارنة فى أى من المجموعتين التجريبية أو الضابطة. وقد تمت مناقشة طريقة تأثير الداى أيثيل نيتروزامين والبرازى كوانتيل على هذه التغيرات المناعية والدموية فى الفئران.

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*: Dept. of Pathology, Fac. Vet. Med., Assiut Univ.
**: Dept. of Forensic & Toxicology, Fac. Vet. Med., Assiut Univ.
Partially supported by USAID/MOH Egypt, 263-0140.2 Project, SRP grant No 01-03-34.

SUMMARY

Praziquantel, the commonly used anti-schistosomal drug, was investigated for hepatic carcinogenesis promoting potential using Diethylnitrosamine (DEN)-induced hepatic neoplasia in rats as a model in our study. Fity rats were given a daily dose of 5 mg/Kg body weight DEN in drinking water, for 12 weeks, while twenty rats were used as control groups. On the sixth week, preneoplastic changes have been developed. Out of these 50 rats, 20 rats were received praziquantel for 6 successive days at a dose of 30 mg/Kg body weight, in addition to DEN administration. At the beginning of the experiment and at week 12 of the experiment, sera and blood samples were collected from all rats and examined. The average increase of the total immunoglobulins (Igs) in rats received DEN (324% ± 64.45) is about 3 fold in comparison to the control group (100% ± 32.5) This increase is reversed in rats receiving praziquantel (115% ± 33.35). Praziquantel alone has no effect in the total Igs (101% ± 76.3). Additionally, the average of the total RBCs counts and the hematocrite value (PCV) were increased by DEN administration (14.35 ± 1.96, 63.62 + 0.4 respectively), in comparison to the control group (7.6 ± 0.36, 46.6 + 0.72). The effect was reversed by praziquantel (9.45 ± 1.05, 40 + 7). However, praziquantel alone has no effect (7.36 ± 0.20, 38.37 ± 0.11). On the other hand, DEN adminsitration increases hemoglobin (HB) concentration with average (21.33 ± 0.47), in comparison to the control group (16.2 ± 1.2). However, this effect could not be reversed by praziquantel (21 ± 4.49), nor could be produced by praziquantel (14.28 ± 1.18). There is no significant changes in the average counts of WBCs or the differential counts in all groups of rats. The mechanism of action DEN and praziquantal on both the immunological and hematological changes was discussed.

INTRODUCTION

Praziquantel is an anti-schistosomal drug that has been used widely in the last ten years. In hepatosplenic patients

Praziquantel can result in reversibility of the fibrotic lesions and the periportal deposion of collagen *(MORCOS et al., 1985; ANDRADE & GRIMAUD (1986) and EL-BADARY et al. (1988).* Additionally, Praziquantel has been shown to affect both the cellular and humoral immune response in schistosoma-infected patients *(SHAKER et al., 1987).* The effect of Praziquantel on the immune response of other associated diseases in schistosoma-infected patients is unclear. One of the major diseases associated with schistosoma infection is viral hepatitis. About one third of schistosma-infected patients have also viral hepatitis *(LYRA et al., 1976).* Moreover, viral hepatitis is considered one of the common complications of hepatic carcinoma *(LI et al., 1991).* This study investigates the effect of Praziquantel on the development of chemically-induced hepatic carcinoma, and the associated humoral immune response. Diethylnitrosamine (DEN) was used in the this study to induce hepatic carcinoma in rats as a model *(MAGEE and FARBER, 1962).* Preneoplastic and early neoplastic changes of liver were experimentally induced. Serological, hematological changes were examined in the presence and absence of Praziquantel administration.

MATERIAL and METHODS

Animals:

The present study was carried out using 70 native white male rats of 6 weeks of age (55-70 gm body weight). The animals were supplied by Experimental Animal Research Center, Faculty of Medicine, Assiut University. Each animal was kept separately in a cage with wide-mesh bottom and fed a commercial rat diet. Before treatment, the animals were acclimatized for 2 weeks and examined to be free from common pathogens.

Induction of hepatocarcinogensis:

The rats were randomly divided into 2 groups. The first group included 50 rats and the secod group included 20 rats. Animals of the first group received a daily dose of 5 mg/Kg body weight of diethylnitrosamine (DEN) (obtained from Merck-Schuchardt Cat No 111732) in drinking water freshly prepared as recommended by *YOUSSEF (1981).* Control animals were left untreated. All rats were kept under observation for evidence of abnormal clinical signs. Whole anticoagulated blood and blood sera were taken from the rats before carcinogenic administration and at 12 weeks post-DEN treatment.

Praziquantel Administration:

After 4 weeks of administration of the carcinogen (DEN), a number of 3 rats were sacrificed weekly. The liver was dissected and processed for paraffin section. After confirmation of the occurrence of hepatic preneoplasia *(TAHA, 1992)*, the rest of the animals were divided into 2 subgroups; A and B. Subgroup A (20 rats). continued the administration of the carcinogen by the same route and dose. Subgroup B (20 rats) received Praziquantel (Bayer-LeverKusen- Germany) regimen in addition to the carcinogen (DEN). Praziquantel was given orally in the drinking water in a dose of 30 mg/Kg body weight for 6 successive days *(ZWINGENBERGER et al., 1990)*. Following Praziquantel intake, 3 rats from each subgroup were sacrificed every week and subjected to postmortem examination. The control rats were also subdivied into 2 subgroups, the first subgroup, included 10 rats, and were given Praziquantel at the same route and dose. Animals of the second subgroup were left untreated. Sera were taken from the tail veins of all rats before DEN administration and at 12 weeks (ends of the experiment).

Enzyme-linked immunosorbent assay (ELISA):

ELISA assay was done as described by *ENGVALL and PERLMANN (1971)*. Briefly, ELISA plates (Flow lab., cat No 76-321-05) were coated with 10 ug/ml of affinity purified rabbit anti-rat immunoglobulins (Igs) in coating buffer (0.1 M Bicarbonate Buffer pH 9.5). The plates were incubated at 4C. After 24 hours, the plates were washed with washing buffer (Phosphate buffered Saline {PBS}) (0.01 M phosphate and 0.15 NaCl pH 7.2), 3 times, blocked with 200 ul of blocking buffer (0.5% BSA in washing buffer) and incubated at room temperature. After 1 hour, the plates were washed 3 times with washing buffer, and the different dilution (1/10, and 1/100) of rat sera (of different groups) were added and incubated at room temperature. After 1-2 hours, the plates were washed 3 times, and the anti-rat polyvalent biotin-conjugates (Sigma Cat No B-2016) were added at dilution 1:1000 and incubated at room temperature. After 30 minutes, the plates were washed 4 times in washing buffer, and the Extra-avidin-peroxidase conjugates (Sigma Cat No E-2636) were added at dilution 1:500, and incubated at room temperature. After 30 minutes, the plates were washed 4 times in washing buffer. The substrate, O-Phenylenediamine (OPD) (Sigma Cat No P-5412) were added at 0.4 mg/ml in substrate buffer (0.05 M phosphate citrate buffer pH 5, and 40 ul fresh H_2O_2/100 ml of substrata buffer). ELISA plates were read at 450 nm, and the mean and standard deviation of the duplicate reading were recorded.

Percent changes of Igs were calculated as follows:

$$\% \text{ changes} = \frac{\text{OD of tested sera}}{\text{OD. control sera}} \times 100$$

Blood Analysis:

Total erythrocytic count (TRBCs), total leucocytic count (TWBCs), and hemoglobin concentration (HB) were determined by using electronic cell counter (cell Dyne 300, Sequoir-Turner) according to *COLES (1980).* Packed cell volume (PCV%) was determined by means of microhematocrite method *(SCHALM, 1979).*

RESULTS

In Fig. 1, the effect of Praziquantel on the total Igs of rats with diethynitrosamine-induced hepatic changes was examined. There is at least three fold increase of the total Igs in the sera of rats after 12 weeks of administration of diethylnitrosamine alone in comparison to the control rats (group 4). This change in the total Igs disappears if Praziquantel is administered with diethylnitrosamine (group 2). Administration of Praziquantel alone (group 3) does not have an effect on the total Igs in comparison to the control rats.

In Fig. 2, the effect of Praziquantel on the total count of WBCs in rats with DEN-induced hepatic neoplasia was stated. The total number of WBCs/mm^3 has not significantly changed with DEN or Praziquantel administration to the control group.

In Fig (3), the effect of Praziquantel on the total count of RBCs in rats with DEN-induced hepatic neoplasia was stated. There is at least two-fold increase of the number of RBCs/mm^3 in the blood of rats 12 weeks after administration of diethylnitrosamine alon in comparison to the control rats (group 4). This change in the number of RBCs/mm^3 disappears if Praziquantel is administered with diethylnitrosamine (group 2). Administration of Praziquantel alone (group 3) does not have an effect on the number of RBCs/mm^3 in comparison to the control rats (group 4).

In Table (1), the effect of Praziquantel on the hemoglobin concentrations, hemaotcrite value, and differential counts have been stated. A significant increase in the HB level has been seen in both groups, 1 and 2 (DEN-induced hepatic neoplasia alone or in combination with Praziquantel) in comparison to the control group (group 4), or rats administered Praziquantel alone(group 3). The increase in HB level in group (2) is not assoicated with increase in the number of RBCs in this group.

At the same time, hematocrite value (PCV) increases in group (1). Administration of Praziquantel to DEN-administered rat group decreased the hematocrite value to the normal level (group 3, and 4). Differential cell counts have not shown any significant changes in the four groups of rats.

DISCUSSION

DEN is an organic nitros compound in which the nitrosogroups attach to the Carbon atom. It is a member of nitrosamines compound family which enters in many industries such as the manufacture of rubber, dyes, lubricating oils, explosives, insecticides, and electric industries. Additionally, nitrosamine compounds could be produced secondarily from canned food (Ender and Ceh (1968) as well as by bacterial organisms (Sander and Seif (1969).

In the acute toxicity, nitrosamine produces centrilobular and midzonal necrosis, depletion of glycogen and fat deposition, as well as dilation of the sinusoidal spaces in the liver. Repeated doses result in liver fibrosis and cirrhosis. The metabolic degradation of the compound by the hepatic microsomal enzymes is an essential process for the toxic mutagenic, teratogenic, and carcinogenic effects of the nitrosamine compounds (HEATH, 1962). Nitrosamine compounds induce hepatocellular carcinoma and cirrhosis of the liver when administered orally in relatively high amounts. With gradual decrease of the dose, fewer hepatocellular carcinomas and more cancer of the oesophagus, pancreas and the urinary bladder were reported *(GOODAL et al., 1968).* Enhanced expression of Ras oncogene (p21) in these tumors have been reported *(fujita et al., 1988). MAGEE and FARBER (1962)* concluded that the acute hepatotoxic effect was caused by the alkylation of proteins and enzymes, while the carcinogenic effect is related to the alkylation of nucleic acids. *THOMAS et al.* (1985) reported the effect of nitrosamine on host resistance and immunity. In that study, Nitrosamines at a dose of 1.5 mg/Kg/day, I.P. for 14 days have no effect on both humoral and cellular response, however, if the dose increases to 5 mg/Kg/day, I.P. for 14 days, there is a 90% decrease in the T-dependent antibody response, while the delayed type hypersensitivity (DTH) response increases by 30%.

On the other hand, Praziquantel is a selective antihelminthic with trematocidal and cestocidal activity but no apparent activity against higher organisms such as nematodes. Praziquantel binds to specific receptors within the parasite and leads to destruction of the tumgent of the parasite and

contraction of the musculature *(PAX et al., 1978)*. Currently, praziquantel is the durg of choice for treating schistosomiasis in humans *(CAMPBELL, 1986)*.

In this study, the effect of Praziquantel (a routine anti-Schistosomal drug) on the DEN-induced hepatic neoplasia was examined. Previous studies by *THAMAVIT et al. (1992)* indicated lack of modulation effects of Praziquantel on DMN-induced lesion development in the Syrian hamster liver. In that study, Praziquantel was given at (200 mg/Kg body weight) 11 times at 2 week intervals starting at week 4 after initial 20 mg/Kg dimethylnitrosamine (DMN) I.P injections at weeks 0 and 2. Sacrification was done at week 28. In another study by *SHIRAI et al. (1991)*, rats were given single I.P. injection of DEN (200 mg/Kg), and after 2 weeks, praziqunatel was given in the diet at concentration 1.5 or 0.5% in the diet, or at 1500 mg/Kg body weight intragastrically. Positive foci of preneoplasia was significantly increased in terms of both number and area with the 1.5% dose, while only area was affected by 0.5% dose.

In our studies, DEN has been used at a daily dose of 5 mg/Kg body weight for 12 weeks. After hepatic preneoplastic changes (4 weeks post-DEN administration), praziquantel was given at a dose of 30 mg/Kg body weight for 6 sucessive days. This dose of praziquantel is comparable to the dose used in the treatment of Schistosomiasis in human (40 mg/Kg). Data in our studies indicated that praziquantel has reduced the incidence of hepatocellular neoplasia *(TAHA, 1992)*, and reverse the effect of DEN on total Igs and both the total RBCs and PCV value but not on the HB concentrations. This controversial result could be explained by the use of different carcinogens. Besides, the dose and the regime of praziquantel are different. Our data also shown that DEN administration increases the level of total Igs, total RBCs, the total PCV counts, and HB values. The mechanism of these changes are not known, but direct stimulatory effect on the bone marrow cells is a possible explanation and needs further investigations. Praziquantel selectively reverses the effects of DEN on the total Igs, the RBCs value, but not on the HB concentration. The mechanism of this selective action of praziquantel might be due to its selective effect on prognoitor cells of the bone marrow. Additinoally, the selective effect of praziquantel on the number of RBCs (group 2), but not the HB level, could be explained by eihter the increased size of RBCs or the increased HB level inside the RBCs in group (2) in comparison to group (1).

Furthermore, the present studies shown that the total Igs has significantly increased (300%) in DEN-administered group in comparison to the control group. The increase in the total Igs might be used as an early sign of pre-neoplastic changes in the DEN-induced hepatic neoplasia model. Evaluation of such sign in early detection of DEN-induced neoplasia in underway.

LEGENDS

Fig. 1: The effect of praziquantel on the total immunoglobulin level of rats with or without DEN-administration. Rats were divided into 4 groups:
Group (1) 20 rats received DEN for 12 weeks.
Group (2) 20 rats received DEN for 12 weeks and Praziquantel on the 6^{th} week for 6 successive days at a dose of 30 mg/Kg body weight.
Group (3) 10 rats received Praziquantel alone on the 6^{th} week for 6 successive days at a dose of 30 mg/Kg body weight.
Group (4) 10 control rats received nothing.

Sera were collected from the four groups of rats at the beginning of the experiment and at the 12^{th} week of the experiment. ELISA were done on the collected sera to estimate the level of total immunoglobulins as described in the material and method. Percent changes of Igs was calculated in comporison to the control group.

Fig. 2: The effect of praziquantel on the total counts of WBCs/mm^3 were done as described in material and method.

Fig. 3: The effect of praziquantel on the total counts of RBCs/mm^3 were done as described in material and method.

Table 1: The effect of praziquantel on the hemoglobin concentration hematocrite value (PCV), and differential leucocyte counts in the four groups of rats as described in Fig. 1. Standard deviation are between brackets.

REFERENCES

Andrade, Z.A. and Grimaud, G.A. (1986): Evalution of Schistosomal hepatic lesions in mice after curative chemotherapy. Am. J. Pathol., 124: 59-65.

Campbell W.C. (1986): The Chemotherapy of parasitic infections. J. of Parasitiology 72: 45-61.

Coles, E.H. (1980): Veterinary Clinical Pathology, 3rd ed. W.B. Saunders Company, Philadelphia, London, Toronto.

El-Badrawy, N.M., Hassanein, H.I., Botros, S.S., Nagy, F.M. Abdella, N.M., and Hegage D. (1988): Effect of Praziquantel on hepatic fibrosis in experimental Schistosomiasis mansoni. Experim. Molec. Pathol., 49: 151-160.

Ender F., and Ceh L. (1968): Occurence of nitrosamines in foodstuffs for human and animal consumption. Food Cosemt. Toxicol. 6: 569-573.

Engvall E., and Perlmann P. (1971): Enzyme-Linked immunosorbent assay (ELISA). qunatitive assay of immunogolubin G. Immunochemistry 8: 871-874.

Fujita, J., Ohuchi, N., Ito, N., Reynolds, S.H., Yoshida, O., Nakayana, H., and Kitamura, Y. (1988): Activation of H-ras oncogene in rat bladder tumors induced by N-butyi-N-(4-hydroxybutyl)-nitrosamine. J. Natl. Cancer Inst. 80 (1): 37-43.

Goodal, C.M., Lijinsky, W., and Tomatis, L. (1968): Tumorigenicity of N-nitroshexamethyleneamine. Cancer Res. 28: 1217-1225.

Heath, D.F. (1962): The decomposition and toxicity of dialkynitrosamines. Biochem. J. 85: 72.

Li, Z.S., Luo, D.D., Dai, J.Z., Zhang, L.L., Wang, X.H., Liu, S.C., Shang. G.F., Yang, Y.X., and Wang, H.C. (1991): Immunohistochemical detection of HBsAg and HBcAg in the liver of patients with *Schistosoma Japonica* complicated by hepatocellular carcinoma. J.Tongji Med. Univ. 11(3): 141-144.

Lyra, L.D., Reboucas G., and Andrade Z.A. (1976): Hepatitis B surface antigen carrier state in hepatosplenic schistosomiasis.Gastroenterology 71: 641-645.

Magee, P.N., and Farber, E. (1962): Toxic liver injury and carcinogenesis: Methylation of rat liver nucleic acids by dimethylnitorsomaine in vivo. Biochem. J. 83: 114-121.

Morcos, S.H., Khayyal, M.T., Mansour, M.A., Saleh, S., Ishak, A., Girgis, N.I., and Dunn M.A. (1985): Reversal of hepatic fibrosis after praziquantel therapy of murine schistosomiasis. Am. J. Trop. Med. Hyg. 34: 314-321.

Pax, R.A., Benneth, J.L., and Fetterer, R. (1978): A benzadiazepine derivative and praziquantel: Effects on musculature of *Schistosoma mansoni and Schistosoma Japonicum.* Nauyn-Schmiedeberg's Archieves of Pharm. 304: 309-315.

Sander, J., and Seif F. (1969): Bakterielle Reduktion von Nitrit im Magen des Menschen als Ursache einer Nitrosamine-Bildung. Arzneimittelforsch 19: 1091-1100.

Schalm, O.W. (1979): Veterinary hematology, 4th ed Lee & Feiberger, Philadelphia, USA.

Shaker, Z.A., Hassanein, H.L., Kamel, M.M., El-Bahairy, N.M., El Kalouby, A.H., and Raziky, E.H. (1987): Effects of Praziquantel on certain immune responses of schistosomiasis egyptian patients. Parasitol. Res. 73: 328-333.

Shirai, T., Jang, K.D., Hakoi, K., Thomavit, W., Pairojkul, C., Hoshiya, T., Hasegawa, R., and Ito, N. (1991): Promotion of heptocarcinogenesis by Praziquantel. Jpn. J. Cancer. Res. 82 (10): 1082-1088.

Taha, M. (1992): Modulating effect of Praziquantel chemotherapy on early carcinogenesis in Rats. Egypt. J. Comp. Path. Clin. Pathol. 5 (2): 343-361.

Thamavit, W., Moore, M.A., Ruchirawat, S., and Ito N. (1992): Lack of modulation effects of Praziquantel on DMN-induced lesion development in Syrian hamster liver. Cancer Lett. 61 (3): 229-232.

Thomas P, Fugmann R., Aranyi C., Barbera P., Gibbons R., and Fenters J. (1985). The effect of dimethylnitrosamine on host resistance and immunity. Toxicol. Appl. Pharmacol. 77 (2) 219-229.

Youssef, N. (1981): The effect of diethylnitrosamine and progesterone on rats with special reference to tumour induction. Ph. D. Thesis submitted for the Departement of Pathology, Faculty of Vet. Medicicine, Assuit University.

Zwingenberger, K., Richter, J., Vergett, J.G., and Feldmeier H. (1990). Praziquantel in the treatment of hepatosplenic schistosomiasis: Biochemical disease markers indicate deacceleration of fibrogenesis and diminution of portal flow obstruction. Trans. R. Soc. Trop. Med. Hug. 48 (2): 252-256.

Table (1):

The effect of Praziquantel (PZQ) on the hemoglobin concentration (HB), hematocrite value (PCV), and differential leucocyte counts in rats administered with Diethylnitrosamine (DEN) or control rats.

(Mean values ± S.E.)

Assay	Drug Administrations			
	DEN	DEN+PZQ	PZQ	Control
1. HB conc. (gm%)	21.33±0.47	21±4.94	14.28±1.18	16.2±1.2
2. PCV (%)	63.62±0.4	40±7	38.37±0.11	46.6±0.72
3.Differential leucocyte counts				
a. Lymphocytes (%)	72±5.6	65.63±2.12	71±4.6	81.2±1.3
b. Neutrophils (%)	24±4.2	32.3±1.02	27.5±4.8	15.2±0.6
c. Monocytes (%)	2.5±1.06	1.5±0.12	4±0.01	2.2±0.31
d. Basophil (%)	0	0	0	0

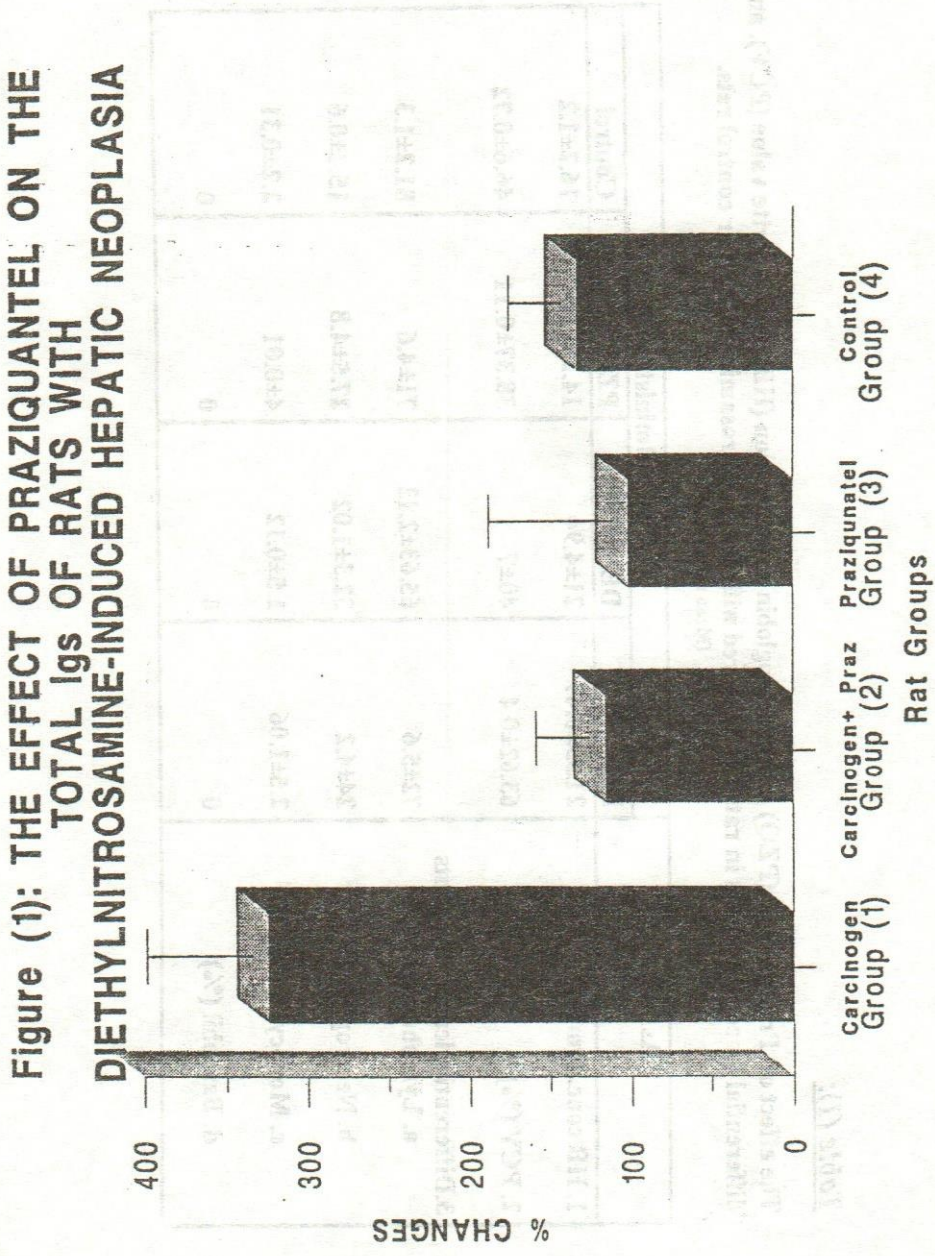
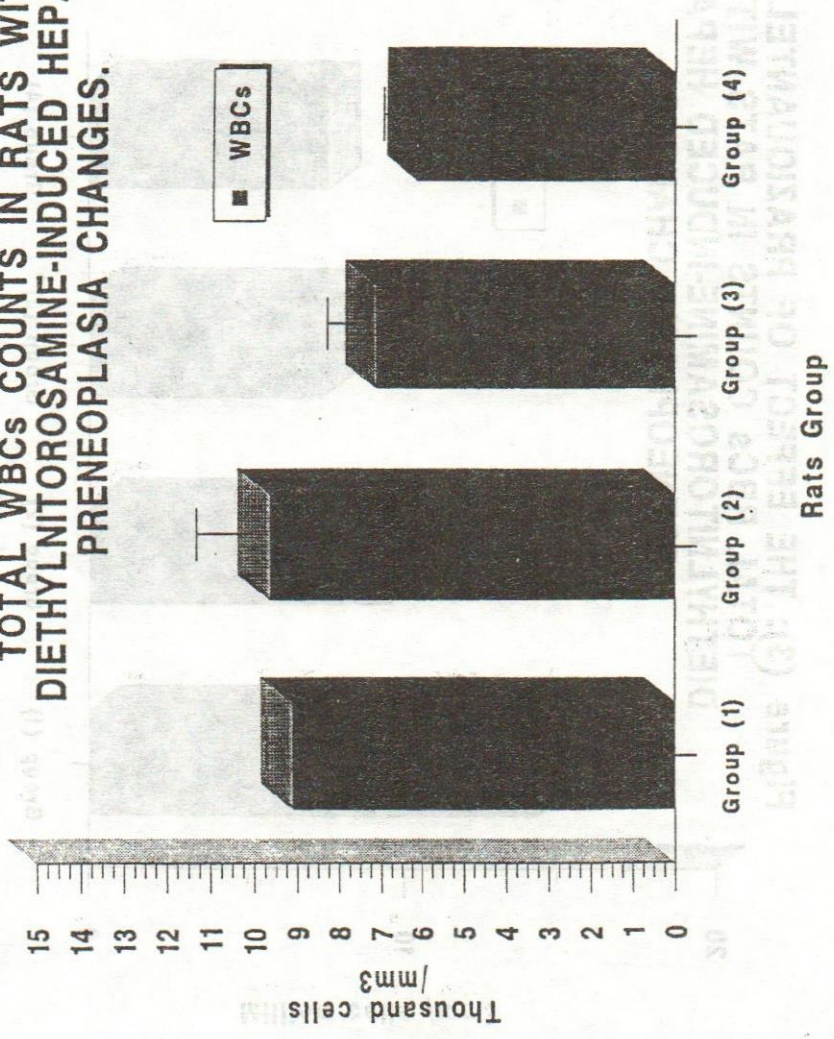


Figure (1): THE EFFECT OF PRAZIQUANTEL ON THE TOTAL Igs OF RATS WITH DIETHYLNITROSAMINE-INDUCED HEPATIC NEOPLASIA
% CHANGES
400
300
200
100
0
Carcinogen Group (1)
Carcinogen+ Praz Group (2)
Praziquantel Group (3)
Control Group (4)
Rat Groups

Figure (2): THE EFFECT OF PRAZIQUANTEL ON THE TOTAL WBCs COUNTS IN RATS WITH DIETHYLNITOROSAMINE-INDUCED HEPATIC PRENEOPLASIA CHANGES.



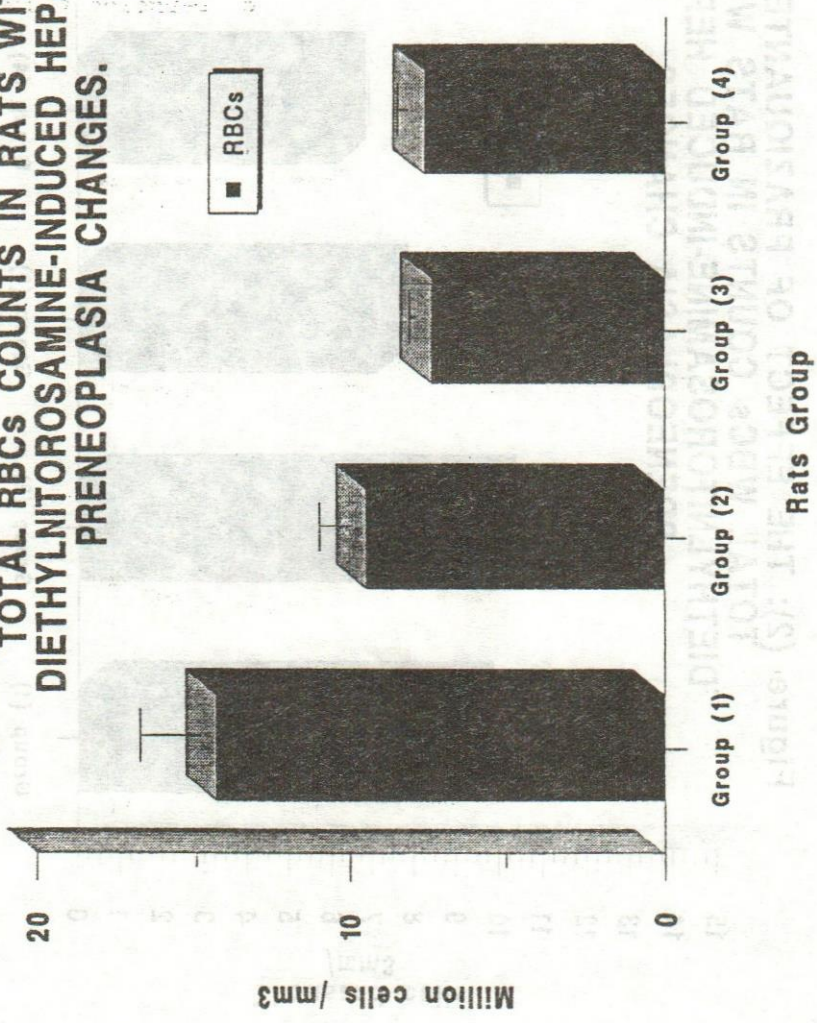


Figure (3): THE EFFECT OF PRAZIQUANTEL ON THE TOTAL RBCs COUNTS IN RATS WITH DIETHYLNITOROSAMINE-INDUCED HEPATIC PRENEOPLASIA CHANGES.