

Spermatogenesis in mice exposed to hydroxyurea : protective effect of natural apple cider vinegar

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الخلاصة

درس تأثير الهيدروكسي يوريا بجرعة 80 ملغم/كغم/يوم عن طريق الفم في عملية تكوين النطف في الفئران البالغة والدور الوقائي لخل التفاح الطبيعي ولوحظ انخفاض في عدد الخلايا الجرثومية الذكرية مع انخفاض معنوي في عدد خلايا النطفية الأولية وارومات النطف في الحيوانات المعاملة لمدة (7) ايام بالهيدروكسي يوريا . وبينت الدراسة ان اعطاء خل التفاح الطبيعي (20ملغم/كغم/يوم) مدة (7) ايام بعد المعاملة بالهيدروكسي يوريا احدث انخفاضاً معنوياً في الخلايا الجرثومية الذكرية والخلايا النطفية الأولية وارومات النطف . وعند اعطاء خل التفاح الطبيعي مع الهيدروكسي يوريا حدثت زيادة معنوية في اقطار النبيبات المنوية والخلايا النطفية الأولية مع زيادة في عدد الخلايا الجرثومية الذكرية . في حين لم يرجع عدد ارومات النطف الى الحد الطبيعي . وتشير النتائج الى التأثير المثبط للهيدروكسي يوريا في عملية تكوين النطف فيما توضح التأثير الوقائي لخل التفاح الطبيعي في العملية ذاتها من تأثيرات الهيدروكسي يوريا .

ABSTRACT

The effect of hydroxyurea at a dose of 80mg/kg/day orally for (7) days on spermatogenesis and the protective role of NACV(Natural apple cider vinegar) has been studied in mature male mice. Hydroxyurea caused a reduction in the number of spermatogonia and a significant decrease in primary spermatocytes and spermatids. Administration of natural apple cider vinegar (20mg/kg/day) for (7) days after hydroxyurea caused a significant decrease in spermatogonia, spermatocytes and spermatids. The administration of natural apple cider vinegar with

hydroxyurea produced a significant increase in the diameter of seminiferous tubules and spermatocyte count and an increase in spermatogonia count, while spermatid count did not return to normal. The present data indicate that hydroxyurea exerts inhibitory action on spermatogenesis, and natural apple cider vinegar has a protective effect against the damaging effect of hydroxyurea .

INTRODUCTION

The production of sperms is an organizing process throughout the reproductive life of the male . The spermatogonia mature into primary spermatocytes and then undergo meiotic division , they divide into secondary spermatocytes and then into spermatids which contain haploid number(23) of chromosomes (1). Recent studies clearly demonstrated that hydroxyurea, an analogue of urea, has inhibitory action on spermatogenesis (2,3). The mechanism of action of hydroxyurea involves inhibition of deoxyribonucleic acid (DNA) synthesis , it exerts its lethal effect on cells in (S) phase by inhibiting the enzyme ribonucleotide reductase (4). The cytotoxicity of hydroxyurea currently used to combat various cancers, sickle cell anemia and human immunodeficiency infection. Exposing decidualized and pregnant uteri of rats to this drug lead to inhibition of cellular components DNA, protein , nitric oxide synthase and decidual prolactin-related protein mRNA (5). Since natural apple cider vinegar (NACV) has hypolipidemic and antioxidative effects (6), and protective effect on bone marrow from depressive effect of hydroxyurea (7),therefore , the present study was conducted to investigate the protective effect of (NACV) on spermatogenesis in mice exposed to hydroxyurea.

MATERIALS AND METHODS

Twenty four male Swiss mice weighting (25-30) g were housed under controlled conditions of natural light (14hrs light and 10hrs dark) and temperature (25 ± 2 °C) . Commercial pelleted food and water were given *ad libitum* .

Experimental Design

Animals were randomly divided into (4) groups (6 mice each).

Group I: It received 0.2 ml of distilled water by oral intubation.

Group II: 80 mg/kg of hydroxyurea was administered to mice by oral intubation for (7) days.

Group III: 80 mg/kg of hydroxyurea was administered to mice by oral intubation for (7) days followed by 20 mg/kg of NACV (seaford, NewYork, 11703) administered by oral intubation for (7) days.

Group IV: 20 mg/kg of NACV and 80 mg/kg of hydroxyurea were administered to mice at the same time by oral intubation for (7)days.

On completion of experiments , animals were sacrificed by ether administration . The testes were quickly removed trimmed and fixed in formal saline. Fixed testes were sectioned at (6 μm) mounted and stained with haematoxylin and eosin. Fifty seminiferous tubules at the same stage were analyzed for each group. The mean diameter of the seminiferous tubules was measured using ocular micrometer . The mean number of spermatogonia, primary spermatocytes and spermatids per tubular cross section were counted. Data were analyzed statistically using one way analysis of variance (ANOVA) and groups difference were determined using Duncan multiple range test. The result were expressed as mean \pm standard error. $P < 0.05$ was considered as statistically significant (8).

RESULTS

Table (1) shows the mean diameter of the seminiferous tubules and the mean number of spermatogonia and primary spermatocytes and spermatids in different animal groups. Hydroxyurea produced a significant ($P < 0.05$) decrease in the mean number of primary spermatocytes and spermatids when compared with the control, while the mean number of spermatogonia did not show significant differences as compared with the control. Natural apple cider vinegar administered for (7) days to mice pretreated for (7) days with hydroxyurea significantly ($P < 0.05$) decreased the mean number of primary spermatocytes as compared with hydroxyurea-treated group and control. The mean number of spermatogonia and spermatids show significant ($P < 0.05$) decrease as compared with the control. The natural apple cider vinegar and hydroxyurea administered to mice at the same time for (7)days produced a significant ($P < 0.05$) increase in the mean diameter of the seminiferous tubule and primary spermatocyte as compared with hydroxyurea-treated group and control. The mean number of spermatogonia returned to the control, while the mean number of spermatids still less than the control level although the difference is nonsignificant.

DISCUSSION

The results of the present study demonstrated that hydroxyurea produced a significant reduction in the number of primary spermatocyte and spermatids and decreased the number of spermatogonia. These results were in agreement with those reported by Wiger *et al.* (2) and Garozzo *et al.* (3) and is clinically manifested in human by azoospermia. The reproductive toxicity of hydroxyurea was apparently related to its inhibitory effect on DNA synthesis in the reproductive cells of the testes (4). Administration of (NACV) to hydroxyurea treated mice after establishment of the above mentioned changes produced no improvement in spermatogenesis, indicating that (NACV) can not provide protection to the damaging effect of hydroxyurea on germ cells in the testis.

The combined administration of (NACV) with hydroxyurea improved significantly primary spermatocyte count and the diameter of the seminiferous tubule. The count of spermatogonia returned to the normal spermatid count did not return to normal and this could be explained by the fact that spermatogenic cycle in mouse requires about (34) days and there was no chance for new spermatids to develop during the course of experiment(7 days) (9).

It is concluded that the administration of (NACV) with the neoplastic agent, hydroxyurea, improved spermatogenesis,(NACV) has repairing effect and preventing cellular DNA from damage by chemical substances.

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Table (1) Effect of natural apple cider vinegar on the mean diameter of seminiferous tubule, mean number of spermatogonia, primary spermatocytes and spermatids per tubular cross section in male mice treated with hydroxyurea .

Experimental Group	Mean diameter of the seminiferous tubules (μm)	Mean number of spermatogonia per tubular cross section	Mean number of primary spermatocytes per tubular cross section	Mean number of spermatid per tubular cross section
Control	125.87 \pm 3.07	40.24 \pm 1.33	92.71 \pm 5.22	28.33 \pm 2.75
hydroxyurea (80 mg/kg) for (7) days	127.09 \pm 1.53	37.69 \pm 2.57	61.19 \pm 5.17*	15.56 \pm 2.54*
hydroxyurea (80 mg/kg) for (7) days followed by natural apple cider vinegar (20 mg/kg) for (7) days	130.78 \pm 2.76	25.72 \pm 0.92*	42.0 \pm 4.6 ^{+*}	14.35 \pm 4.93*
apple cider vinegar (20 mg/kg) + hydroxyurea (80 mg/kg) for (7) days	147.36 \pm 2.45 ^{+*}	42.37 \pm 1.41	119.22 \pm 4.29 ^{+*}	17.39 \pm 3.96

Values are mean \pm S.E

* Significantly different from control (P<0.05)

+ Significantly different from group II (P<0.05)