

Jihad FAKHOURI<sup>1</sup>, Riad SARKIS<sup>2</sup>, Myrna CHABABI-ATALLAH<sup>3</sup>, Georges AFTIMOS<sup>3</sup>

Fakhouri J, Sarkis R, Chababi-Atallah M, Aftimos G. Toxic effects of methyl methacrylate monomer on male genital tissues. *In vitro* study in rats. *J Med Liban* 2008 ; 56 (1) : 22-26.

**ABSTRACT • INTRODUCTION :** Methyl methacrylate (MMA), a widely used monomer in dentistry and medicine has been reported to cause abnormalities or lesions in several organs. Experimental and clinical studies have documented that monomers may cause a wide range of adverse health effects such as irritation to skin, eyes, and mucous membranes, allergic dermatitis, stomatitis, asthma, neuropathy, disturbances of the central nervous system, liver toxicity, and fertility disturbances.

**OBJECTIVES :** The purpose of this study was to determine whether MMA mixed with water at four different concentrations is able to affect the histological structure of testicular tissues and seminal vesicle on male rats.

**METHODS :** The target population consisted of 60 male Sprague-Dawley rats. They were housed in colony cages and divided into five groups : The first group (n = 15) designated as the control group and four experimental groups (n = 45). Experiments were conducted by exposing the four experimental groups to MMA administered *per os* mixed with water at different concentrations (4%, 8%, 16%, 32%). The exposure duration was eight months. The testicles and the seminal vesicles were then extracted, dissected, fixed in Bouin liquid fixative and were submitted to the pathology laboratory (National Institute of Pathology) for histopathological examination.

**RESULTS :** Seven out of 10 rats to which the MMA was administered at a concentration of 32% showed partial seminal vesicle atrophy. The seminal vesicles in the remaining rats showed normal histology in all specimens. Testis, epididymis and vas deferens showed normal histology in all rats.

**CONCLUSION :** The data in this study showed that MMA administered at high concentration is associated to seminal vesicle atrophy. These findings let us suggest that this effect could be the result of either a direct effect of MMA on testosterone levels (as shown in our first study), or through its possible action on other organs involved in testosterone metabolism and seminal vesicle trophicity such as the hypophysis.

Fakhouri J, Sarkis R, Chababi-Atallah M, Aftimos G. Effet toxique du monomère méthacrylate de méthyle sur les tissus génitaux mâles. Étude *in vitro* chez le rat. *J Med Liban* 2008 ; 56 (1) : 22-26.

**RÉSUMÉ • INTRODUCTION :** Le méthacrylate de méthyle (MMA) est très souvent utilisé en médecine et en dentisterie. Son implication dans les irritations dermiques et oculaires, les dermatites, les stomatites, l'asthme et surtout son effet toxique sur le foie, le système nerveux central et la fertilité a été prouvée et confirmée par de nombreux auteurs.

**OBJECTIF :** Étudier l'effet du MMA dilué à l'eau administré par voie orale sur l'état histologique du testicule et de la vésicule séminale chez le rat.

**MÉTHODES :** Soixante rats mâles Sprague-Dawley ont été utilisés et divisés en 5 groupes. Le 1<sup>er</sup> groupe (n = 15) étant le groupe témoin et 4 groupes (n = 45), ceux de l'expérimentation. De l'eau mélangée avec du MMA a été administrée à 4 différentes concentrations (4%, 8%, 16% et 32%). La durée de l'exposition a été de 8 mois. Les testicules et les vésicules séminales ont été prélevés, disséqués et fixés par le liquide de Bouin et soumis au laboratoire d'anatomo-pathologie (National Institute of Pathology) pour des examens histologiques.

**RÉSULTATS :** Sept des 10 rats du groupe V qui ont été exposés au MMA à une concentration de 32%, ont présenté une atrophie partielle des vésicules séminales. Les vésicules séminales des autres rats n'ont montré aucune anomalie. Les testicules, les canaux déférents de l'ensemble des rats n'ont pas montré de pathologies.

**CONCLUSION :** Les résultats de cette étude montrent que l'administration d'une importante concentration de MMA est associée à une atrophie de la vésicule séminale. Ceci pourrait être expliqué aussi par l'action directe du MMA sur la testostérone ou bien son action sur d'autres organes, tels que l'hypophyse, impliqués dans le métabolisme de la testostérone et dans l'atrophie de la vésicule séminale.

## INTRODUCTION

In reconstructive orthopedic surgery, methyl methacrylate (MMA) toxicity was evidenced as a perioperative cardiorespiratory dysfunction and postoperative deep vein thrombosis, in particular at the site of cemented

Saint-Joseph University, Beirut, Lebanon, Departments of <sup>1</sup>Removable Prosthodontics, Faculty of Dental Medicine ; <sup>2</sup>Surgical Sciences Research, Faculty of Medicine ; <sup>3</sup>Histopathology, Faculty of Medicine & National Institute of Pathology (INP).

Corresponding author : Jihad Fakhouri, DDS. Faculty of Dental Medicine. Saint-Joseph University. Beirut. Lebanon.

Phone : +961 3 75 85 72 Fax : +961 4 98 42 98

e-mail : [jihad.fakhouri@usj.edu.lb](mailto:jihad.fakhouri@usj.edu.lb) OR [riad.sarkis@usj.edu.lb](mailto:riad.sarkis@usj.edu.lb)

areas (Stamatakis et al. 1977, Rinecker 1980, Ferris and Kinsella 1984, Dahl et al. 1988, 1992, Lynch et al. 1988, Duncan 1989, Francis et al. 1989, Hogevoold et al. 1990) [1-9].

Dahl et al. investigated the influence of MMA on the cellular integrity of endothelial cells and found that all cells treated with 10 µg/ml showed marked signs of cytotoxicity. After one minute incubation, the endothelial cells lost their normal polygonal shape and spread-out appearance, the cells lost their adherence to one another and to the substratum, rounded up and were eventually shed from surface, leaving only remnants of the basal cell membrane behind. Exposure of monocytes to MMA (10 µg/ml) resulted in immediate cell enlargement and loss of membrane folding. This was followed by shedding of membrane vesicles or fragments, membrane disintegration and total cellular disorganization affecting the majority of cells after 30-min incubation [9].

Granulocytes exposed to MMA (10 µg/ml) showed immediate retraction of pseudo-filopodia that had evolved as a result of glass adherence and caused the formation of large membrane blebs. The numerous delicate membrane protrusions of normal granulocytes disappeared rapidly (*1 min exposure*) as the cell became distended and acquired a smoother surface. Membrane dissolution and exposure of cytoplasmic constituents evolved, and after 30 min of exposure, most of the cells were totally destroyed.

The physical and chemical properties of MMA appear to affect the lipid bilayer in biological membranes, when cytotoxicity testing was carried out against primary human gingival fibroblasts, human submandibular gland adenocarcinoma cell line, and human erythrocytes (Chey et al. 2005) [10].

Concerning MMA potential in carcinogenesis, the International Agency for Research of Cancer (IARC) has concluded that there is inadequate evidence for the carcinogenicity of MMA in humans and there is evidence suggesting a lack of carcinogenicity of MMA in experimental animals. The overall evaluation concluded that MMA would not be classified as carcinogenic [11].

The lung was found to be a major site of injury after methyl methacrylate exposure, but changes were also reported in the liver, kidney and heart after higher concentrations (Hems et al. 1966) [12]. More recent papers indicate that exposure of animals to MMA vapor has a profound effect also on the central nervous system (Corkill et al. 1976) [13].

Contradictory results have been reported concerning histological studies of MMA toxicity on internal organs. Some authors reported that most characteristic observations in animals exposed to MMA vapors were degenerative changes in the liver (Blanchet et al.) [14]. On the other hand, Borzelleca et al. while testing chronic oral toxicity of MMA in rats and dogs, failed to find histological lesions in all tissues tested [15].

Singh et al. reported that the administration of MMA to pregnant rats produces a dose-related increase in

skeletal abnormalities, and a reduction in fetal weight at birth [16].

However, so far there is no study indicating direct implication of the MMA on fertility mechanism.

## OBJECTIVES

All these contradictory reports and the present, widespread usage of MMA monomer in medicine and dentistry led us to study whether the mixture of MMA with water at four different concentrations is able to affect the histological structure of testicular and seminal vesicle tissues on male rats.

## MATERIALS AND METHODS

### Animals

Sixty Sprague-Dawley rats weighing between 220 and 400 g were used. All rats were examined by a qualified veterinary and all of them were free of any disease before the experimentation. They were equilibrated for 8 months and were housed in closed colony cages under controlled conditions of temperature and illumination.

### Conditions of exposure

The rats were randomly divided into five groups, Group I (A, B, C) (n = 15) consisted of control animals, Group II (D, E, F) (n = 15) was exposed to MMA mixed with water at a concentration of 4‰ (v/v), Group III (G, H) (n = 10) was exposed to MMA mixed with water at a concentration of 8‰ (v/v), Group IV (I, J) (n = 10) was exposed at a concentration of 16‰ (v/v) and Group V (K, L) (n = 10) at 32‰ (v/v).

The animals were housed in colony cages (five per cage), so 12 cages were obtained (Group I : 3 cages, Group II : 3 cages, Group III : 2 cages, Group IV : 2 cages, Group V : 2 cages).

In each cage, and in order to identify each of the 5 rats, perforations were made in their ears. One rat underwent a single perforation on the right ear (<sub>1</sub>RE), the other one had a single perforation in left ear (<sub>1</sub>LE). The third, had two perforations in right ear (<sub>2</sub>RE), the fourth had two perforations in the left ear (<sub>2</sub>LE) and the fifth had one perforation in each ear (<sub>1</sub>RE <sub>1</sub>LE).

Temperature (*between 20°C to 23°C*), humidity (*between 50% to 70%*) in the animal room were automatically controlled as well as daylight timing of 9 hours which are a must for optimal health conditions and survival of the rats.

The daily food intake was 25 grams per rat consisting of 18% protein, 3% fat, 5.5% cellulose and 7.5% ash (*Crispy rat : Verselle-Laga Belgium*).

At 8 months, which was the exposure duration, rats were anaesthetized and sacrificed. Dissection concerned the testicles and the seminal vesicles deferens canals that were immediately immersed in Bouin fixative. They were then sent to the pathology laboratory (National Institute of Pathology) and were processed for microscopy.

### Assay methods

After fixation, the above organs were cut according to their major axis which varied between 1.4 cm and 2 cm. Samples were taken from each organ and processed in an automated machine (*Ventana Renaissance automaton*) to undergo dehydration, clearing and paraffin infiltration. Tissue specimens were then retrieved from the machine, embedded in paraffin blocks and then cut with microtome at 4 µm of thickness. Histologic slides were stained with haematoxylin and eosin (*H & E*) for light microscopic examination.

## RESULTS

### Seminal vesicles

Histological examinations of the seminal vesicles showed partial atrophy of the lining epithelium where it became cubic or flattened. The lumen was dilated, usually empty compared to the normal areas where it was filled with dense and acidophilic secretions. These anomalies involved the specimens L<sub>1</sub>RE, L<sub>2</sub>RE, L<sub>2</sub>RE, K<sub>1</sub>RE, K<sub>1</sub>LE, K<sub>2</sub>RE, K<sub>2</sub>LE and H<sub>1</sub>RE. The seminal vesicles in the remaining rats showed normal histology (Fig. 1).

In normal state, each seminal vesicle is a highly convoluted, unbranched tubular diverticulum of the vas deferens. Seminal vesicle mucosa is composed of a fibroelastic lamina propria thrown up into tall, narrow complicated folds covered by non-ciliated, tall columnar epithelium cells and a population of non-specialized basal round cells. The secretion contains abundant fructose and other sugars, prostaglandins, proteins, amino acids, citric acid and ascorbic acid (Fig. 2).

### Testis

All specimens showed testicular tissue devoid of lesions. The spermatogenesis was preserved and complete. Basement membranes were of normal thickness, and the Leydig cells were normotrophic. There was a slight interstitial edema, presumed artifactual (Fig. 3).

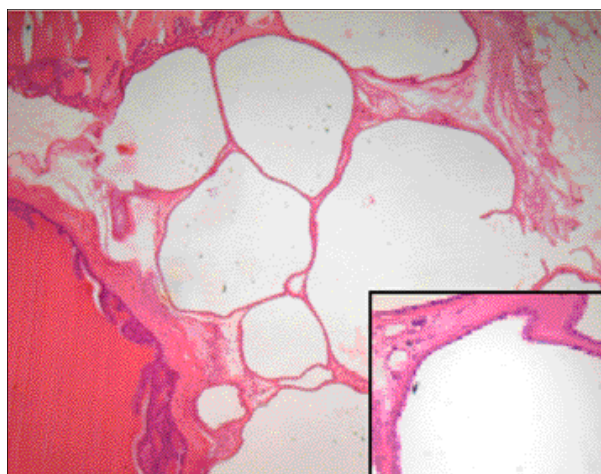
### Epididymis and vas deferens

The epididymis and vas deferens were also devoid of lesion and their lumen was filled with spermatozoa (Fig. 4).

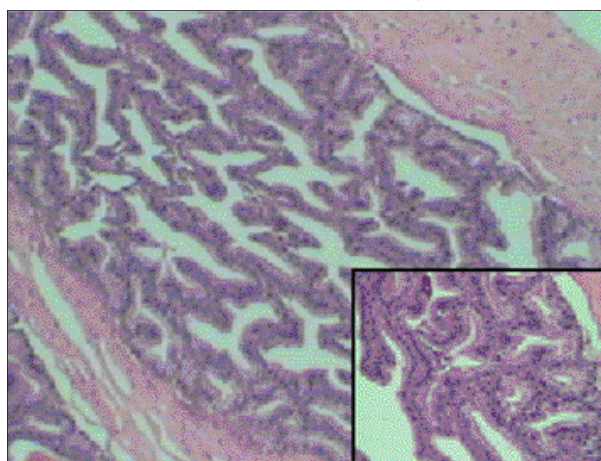
**TABLE I**  
MEAN BODY WEIGHT OF RATS IN EACH GROUP BEFORE THE EXPERIMENTATION

Group	MMA Concentration	Weight (g)
I	Control	268
II	4‰	274
III	8‰	272
IV	16‰	281
V	32‰	282

MMA : methyl methacrylate



**FIGURE 1.** Micrograph of seminal vesicle of L<sub>1</sub>RE showing the atrophy of the epithelium. (HE x 50, inset HE x 100)



**FIGURE 2.** Micrograph of non atrophic seminal vesicle of A<sub>1</sub>RE showing part of the muscular surround, the pattern of tall mucosal folds and the tall columnar lining. (HE x 50, inset HE x 100)

### Correlation between body weight/concentration of MMA/testosterone levels

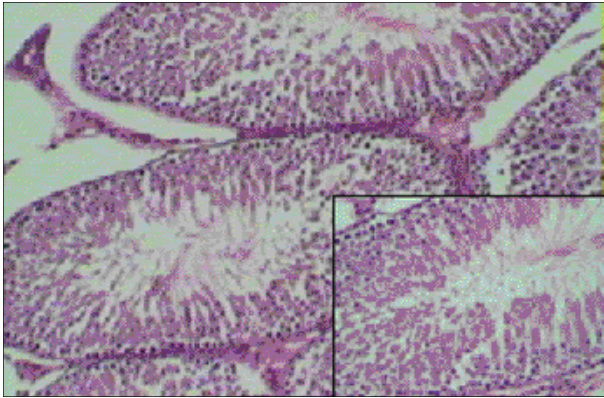
Total body weight of animals randomly assigned to each group at the beginning of the study was not significantly different (Table I).

Eight months later, the body weight of rats, in which the testosterone level has increased, displayed an increase of 38% while all the others showed an increase of their body weight only of 23% (Table II).

## DISCUSSION

In our previous study (see pp. 11-15), we showed that the blood testosterone level depended on the MMA concentration administrated *per os*. At low (4‰) and high (32‰) concentration the testosterone level showed a significant decrease.

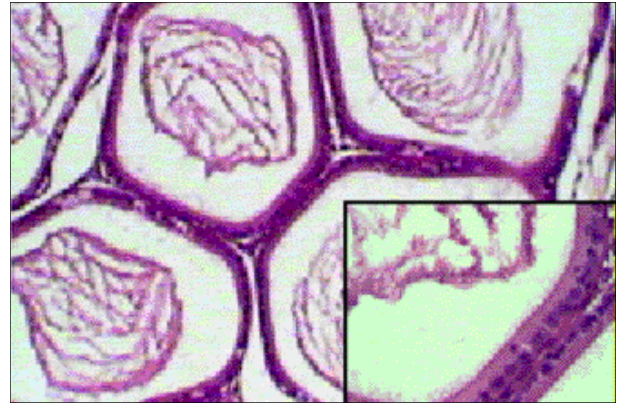
Our histological results showed that 7 out of 10 rats in Group V to which MMA was administrated at a concen-



**FIGURE 3.** Micrograph showing seminiferous tubules cut in transverse, longitudinal and oblique section lined by germinal epithelium and enclosed by tunica propria, in the interstices are blood vessels and clumps of Leydig (interstitial) cells.  
(HE x 50, inset HE x 100)

tration of 32‰ suffered seminal vesicle atrophy. However, the rats in Group II which were given 4‰ of MMA did not show any sign of atrophy despite the fact that their blood testosterone level had also decreased.

According to these results, one would think that the seminal vesicle trophicity seems to be androgen-dependent and the alteration of hormonal status could affect its histological state. However, there is still one question that cannot be answered in this study : how to explain the absence of seminal vesicle atrophy in rats administered low MMA concentration (4‰) despite the decrease level of testosterone. This could be explained, but remains to be proven, by the fact that the low level of testosterone could not be the only cause of seminal vesicle atrophy, and there might be other organs interference with this trophicity, especially hypophysis and/or liver ; knowing that the liver plays a role in the metabolism of MMA as well as of gonadotrophins.



**FIGURE 4.** Low-power and high-power micrograph of epididymal duct lined by tall columnar epithelium with basal nuclei, the lumina contain clumps of spermatozoa.  
(HE x 50, inset HE x 100)

Another finding in our study is that the testosterone seems to interfere not only on seminal vesicle trophicity but also in muscle weight. In fact, the weight of the rats which were subject to a rise in testosterone level increased 50% more than the other rats. Axell et al. showed that the implantation of testosterone implants leads to an increase in animal's muscle weight [17].

By reviewing the literature we found that Axell et al. have demonstrated that orchidectomized mice had significantly decreased muscle and seminal vesicle mass. Seminal vesicle was measured because it is a sensitive biomarker of androgen action. Testosterone treatment abolished the orchidectomy-induced loss of seminal vesicle mass and increased seminal vesicle mass 2.5 fold higher than the orchidectomized mice [17].

El Gohary et al. showed that six days after the implantation of two capsules containing testosterone (30 mg/capsule) in castrated rats provided a seminal vesicle weight equivalent to that of non-castrated rats, while the

**TABLE II**  
BODY WEIGHT OF RATS IN WHICH THE TESTOSTERONE LEVEL HAS INCREASED BEFORE AND AFTER THE EXPERIMENTATION

IDENTIFICATION OF RATS	Testosterone before (ng/ml)	Testosterone after (ng/ml)	Weight before (g)	Weight after (g)
H <sub>1</sub> RE (8‰)	3.22	3.90	325	452
H <sub>1</sub> RE <sub>1</sub> LE (8‰)	3.64	4.12	316	444
I <sub>1</sub> RE (16‰)	1.30	2.30	326	434
I <sub>2</sub> RE (16‰)	1.95	3.50	348	455
I <sub>2</sub> LE (16‰)	1.46	2.30	350	496
J <sub>1</sub> RE (16‰)	1.75	2.50	331	409
J <sub>1</sub> LE (16‰)	1.50	1.90	339	461
J <sub>2</sub> RE (16‰)	0.48	1.31	300	462
J <sub>2</sub> LE (16‰)	0.60	1.67	298	458
K <sub>1</sub> RE (32‰)	0.80	0.95	318	429
L <sub>1</sub> RE <sub>1</sub> LE (32‰)	0.90	1.45	304	415

castration produced significant decrease in weights of the seminal vesicle and the implantation of two empty capsules had no effects on the weight of seminal vesicle in castrated rats indicating that the silicone capsule contained no androgenic agent [18].

Bangalore et al. showed that forty days post-hypophysectomy the seminal vesicle regressed significantly. This regression is accentuated by 85 days post-surgery. This study demonstrated the crucial role of hypophysis in the preservation of seminal vesicle mass [19]. The level of circulating androgens required to maintain seminal vesicle mass is currently not known. It is proved that lower doses of circulating androgens (testosterone, hypophysis hormone) would result in decrease in seminal vesicle mass [20].

### CONCLUSION

We consider that the liver couldn't metabolize the MMA at both high (32%) and low (4%) concentration by its nonspecific carboxylesterase enzyme. The MMA that circulates in the blood is associated to the seminal vesicle atrophy either through its direct action on testosterone secretion or its possible indirect action on testosterone through the hypophysis. This hypothesis is still to be demonstrated by further studies which will address the concomitant effects of MMA on the pituitary gland, the testis and the seminal vesicle, and studying in parallel the MMA given dose, its concentration fluctuation in the blood, the histology of the above mentioned organs as well as their respective hormone blood concentrations.

### REFERENCES

1. Stamakakis JD, Kakkar D. Femoral vein thrombosis and total hip replacement. *Br Med J* 1977 ; 2 : 223-5.
2. Rinecker H. New clinico-pathophysiological studies on the bone cement implantation syndrome. *Arch Orthop Trauma Surg* 1980 ; 97 (4) : 263-74.
3. Ferris BD, Kinsella M. Fatal complications of the surgical treatment of distal fractures of the femur. *Anaesth* 1989 ; 44 (2) : 149-54.
4. Dahl OE, Molnar I, Ro JS. Global tests on coagulation and fibrinolysis in systemic and pulmonary circulation accompanying hip arthroplasty with acrylic cement. *Thromb Res* 1988 ; 50 (6) : 865-73.
5. Lynch AF, Bourne RB, Rorabeck CH. Deep-vein thrombosis and continuous passive motion after total knee arthroplasty. *J Bone Joint Surg* 1988 ; 70 : 11-14.
6. Duncan JA. Intra-operative collapse or death related to the use of acrylic cement in hip surgery. *Anaesth* 1989 ; 44 (2) : 149-52.
7. Francis CW, Pellegrini VD. Prevention of venous thrombosis after total hip arthroplasty antithrombin III and low-dose heparin compared with dextran 40. *J Bone Joint Surg* 1989 ; 71 (3) : 327-35.
8. Hogenold HE, Hoiseth A, Reikeras O. Deep vein thrombosis after total hip replacement. A venographic study. *Acta Radiol* 1990 ; 31 (6) : 571-3.
9. Dahl OE, Pedersen T, Kierulf P, Wetvik AB. Intrapulmonary thrombin generation and its relation to monomethylmethacrylate plasma levels during hip arthroplasty. *Acta Anaesthesiol Scand* 1992 ; 36 : 331-5.
10. Chey Y, Zajac JD, Mac Lean HE. Androgen of satellite cell function. *J Endocrinol* 2005 ; 186 : 21-31.
11. Herbst KL, Bhasin S. Testosterone action on skeletal muscle. *Curr Opin Clin Nutr Metab Care* 2004 ; 7 : 271-7.
12. Hems R, Ross B. Gluconeogenesis in the perfused rat liver. *Biochem J* 1966 ; 101 : 284-92.
13. Corkill J, Lloyd E, Hoyle P. Toxicology of methyl methacrylate in human blood in vitro. *Clin Chim Acta* 1976 ; 68 : 141-6.
14. Blanchet LJ, Bowman DC. Effects of methyl methacrylate monomer vapors on respiration and circulation in unanesthetized rats. *J Prosthet Dent* 1982 ; 48 (3) : 344-54.
15. Borzelleca JF. Studies on the chronic oral toxicity of monomeric ethyl acrylate and methyl methacrylate. *Toxicol Appl Pharmacol* 1964 ; 5 : 29-36.
16. Singh AR, Lawrence HM, Antain J. Embryonic-fetal toxicity and teratogenic effects of a group of methacrylate esters in rats. *J Dent Res* 1999 ; 51 : 1632-8.
17. Axell AM, Mac Lean HE. Continuous testosterone administration prevents skeletal muscle atrophy and enhances resistance to fatigue in orchidectomized male mice. *Am J Physiol Endocrinol Metab* 2006 ; 58 : 126-35.
18. El Gohary M, Cavazos JP. Effects of testosterone on histochemical reactions of epithelium of hamster ductus epididymidis and seminal vesicle. *Intersciences* 2005 ; 144 (3) : 229-37.
19. Bangalore I, Sundararaj S. Effects of castration and hypophysectomy on the seminal vesicles on the catfish, *heteropneustes fossilis*. Department of Zoology, University of Delhi, Delhi 7, India, 2003.
20. Fitts JM, Klein RM, Powers A. Comparaison of tamoxifen and testosterone propionate in male rats. *J Androl* 2001 ; 25 (4) : 26-31.

التأثيرات السمية لميتيل ميتاكريلات احادية القسيم MMA على انسجة الذكر التناسلية. دراسة مخبرية على الجرذان.

موجز: مدخل MMA غالبا ما يستعمل في الطب وفي طبابة الاسنان. وتورطه في سمية الكبد واضطراب الاخصاب ثبت وتؤكد من قبل عدة مستعملين.

الموضوع - دراسة تأثير MMA المعطى ممزوجا مع الماء على انسجة الخصية والحوصل المنوي عند الجرذ. الطرق - ٦٠ جرذا ذكرا Sprague - Dawley استعمل خمسة عشر منها في قصص خاص وهي المجموعة الاولى جعلناها للمقارنة واربع مجموعات (عدد ٤٥) اجريت عليها التجربة. وضع الجرذان في مكان خاص استنادا الى التوصيات العالمية لاختصاصي الحيوان. مزج MMA مع الماء واعطي باربع تركيزات مختلفة ٤ - ٨ - ١٦ - ٣٢ بالالف) وكانت مدة التجربة ثمانية شهور. استؤصلت الخصى والحوصلات المنوية وجرى تشريحها وتثبيتها بسائل Bouin وارسلت الى مختبر دراسة الامراض للفحص النسجي في المعهد الوطني للأمراض.

النتائج - ٧ جرذان من ١٠ من المجموعة (٥) التي تعرضت الى MMA المركز ٣٢ بالالف تبين عندها ضمور جزئي للحوصلات المنوية اما الحوصلات المنوية لبقية الجرذان فلم تظهر اية تغيرات شاذة فيها، الخصى والاقتية النافذة لمجموع الجرذان لم تظهر عندها اية مراضة.

الخلاصة - اظهرت نتائج الدراسات بان اعطاء المركبات الكثيفة لمادة MMA تشترك مع ضمور الحوصل المنوي ويمكن تفسير ذلك بالتأثير المباشر لمادة MMA على التستسترون او التأثير على اعضاء كالنخامة مثلا، متورطة في ايض (استقلاب) التستسترون وضمور الحوصل المنوي.