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Effect of Depot-Medroxyprogesterone Acetate on the Kidney of Rat: A Histological Study

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ABSTRACT

Endogenous natural progestins are essential for the initiation and maintenance of pregnancy but exogenous progestins and their derivatives may induce adverse effect on different organ. The kidney being a vital and highly active organ is affected by most of the drugs. It's the main organ responsible for excretion of the metabolic products of hormones. Therefore the present study aimed to evaluate the effect of injectable contraceptive, Depot-medroxyprogesterone acetate on the kidney of white albino rat. There was significant increase in the diameter of PCT, DCT, renal corpuscle, glomerulus, and Bowman's space of experimental rat kidney. Hemorrhage was seen in cortical and medullary region with lymphocytic infiltration in Bowman's space and necrosis of some renal corpuscles. The study concluded that the more the duration and dose of the DMPA severe the alternation in the histological architecture of kidney.

Introduction

A contraceptive method is one which helps the women to avoid unwanted pregnancy resulting from coitus and there are many method of contraception but the ideal is one, which is safe effective, acceptable, reliable, and requires less medical supervision.[1] Several characteristics of injectable contraceptives have led to their widespread use as they provide a highly effective contraception that last for more than 2 month after a single dose of injection and they do not contain estrogen so, they are free from adverse effect of estrogen.[2] The only injectable contraceptive drugs currently available are Depot-medroxyprogesterone acetate and norethisterone enantate.[3]

Depot-medroxyprogesterone acetate (DMPA) is an aqueous suspension of medroxyprogesterone acetate (MPA), a synthetic analog of 17α-hydroxyprogesterone and variant of the human hormone progesterone.[4] This is also classified as sex hormone binding globulin (SHBG).[5] Depo -Provera, a microcrystalline suspension of medroxyprogesterone acetate (DMPA), is a long-acting, highly effective injectable contraceptive and one of the major means of family planning.[6] The primary mechanism of action is the inhibition of gonadotropins hormone (FSH and LH) thus inhibition of ovulation.[7] It also increases the viscosity of cervical mucus, making the mucus less easily penetrable to sperm.[8] The contraceptive mode is a depot injection containing 150 mg medroxyprogesterone acetate which is administered by intramuscular route at a plasma concentration of about 1ng/ml) given in the gluteal or deltoid muscle within first 5th day of menstruation.[9] DMPA has been favored because it creates amenorrhea and reduces cycling in many patients while simultaneously serving as highly effective contraception which protects against the development of uterine fibroids.[10] It has been used as a contraceptive agent by more than 68 million women in more than 114 countries worldwide.[11] Unlike oral contraceptives, DMPA have been proven to be relatively safe and is free from the adverse effect of estrogen and furthermore, progestogens, unlike estrogens, do not suppress lactation, which is an important consideration for postpartum contraception where infant health is dependent upon breast-feeding.[12] Evidence suggests that progestogen-only injectables are more cost-effective than the combined oral contraceptive (COC) pill even after 1 year of use.[13] Endogenous natural progestins are essential for the initiation and maintenance of pregnancy but exogenous progestin and their derivatives induce adverse effect like delay in the return of fertility.[14]

Other side effects like loss in bone mineral density, Increased total cholesterol (TC), triglyceride (TG), low-density lipoprotein cholesterol (LDL) and decreased of high-density lipoprotein cholesterol (HDL-C) level were seen in DMPA users.[15] Therefore the present study has been undertaken to evaluate the effect of injectable contraceptive, Depot-medroxyprogesterone acetate on the kidney of white albino rat which is the vital organ for the excretion of most of drug including the hormones.

Material and Methods

Sixty healthy female Wistar Albino female rats weighing 150-200 gm were obtained from the animal house of BPKIHS, Dharan. They were given standard pellet diet and drinking water and libitum. They were maintained in a well ventilated room at controlled ambient temperature (25°c) with a 12 hours in alternating light- dark cycle. They were housed in polypropylene cage (40 cm × 25 cm × 15 cm) with the paddy husk bed, which was changed on every 4-5 days.

Preparation of the Depot-Medroxyprogesterone Acetate Solution

DMPA vials sold as 'Sangini' in Nepal are manufactured by **Pfizer pharmaceuticals group**. One vial containing 150 mg/ml suspension was diluted in distilled water. The experimental groups were given DMPA in the doses of 2.4 mg and 5.4 mg intramuscularly per week for 8 and for 12 weeks respectively.

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The control groups were given 0.25 ml and 0.5 ml of normal saline intramuscularly for 8 and for 12 weeks respectively. The doses were converted from human dose to rat dose by using multiplication factors for dose conversion between different species by **Paget and Barnes** as follow.[16]

Drug to be given for rat = 0.018 × Human dose

Experimental Design and Treatment Regimen

Animals were randomly divided into 4 different groups, with in each group *n*=15 rats, Total number *n= 60*

Low dose and high dose animal were sacrificed one day after the completion of 8 and 12 weeks respectively. The rats were anesthetized with Ether soaked in cotton and kidneys were fixed by In Vivo Perfusion method. After completion of perfusion, the kidney were isolated from the body with help of scalpel and forceps and post fixed for 24 hours with Bouin's Fluid. Weight of the kidneys were measured by electronic balance and kidneys thus obtained were cut into pieces of 5 mm to fix in neutral buffered formalin for 7 days and processed for making paraffin blocks. The blocks were trimmed, sectioned at 6 µm thickness and stained by routine H&E (Hematoxylin and Eosin) staining. All sections were examined under light microscope.

Data Entry and Statistics Tool Applied

The data was collected and entered in SPSS 17 (IBM SPSS 17.0 Inc.) and independent – Sample t test was applied to see the level of significant, 95 % CI. $P < 0.05$ was set to demonstrate significant level.

Ethical clearance was taken as per the guideline of Institutional Ethical Review Board (IERB no. 143) of BPKIHS, Dharan, Nepal.

Result

Subjective changes

Change in the weight of the rat and weight of the kidney of rat were measured. The mean value of initial weight of experimental low dose and control rats was (159.67 gm 158.8 gm) respectively. Similarly, the mean value of initial weight of experimental high dose and control rats was (164 gm and 163.67 gm) respectively. The weight of experimental group rat was compared with control group rat.

Group	Sample size	Duration of experiment
Group A – Control	15 healthy female rats	8 week
Group B – Control	15 healthy female rats	12 week
Group C – Experimental low dose	15 healthy female rats	8 week
Group D- Experimental high dose	15 healthy female rats	12 week

Table 1. Weight of kidney of rat.

Parameter (Mean ± SD)	Control *n=15*	Experimental *n=15*	P- value
Weight of kidney of low dose groups	0.520 ± **0**.110	0.647 ± 0.126	0.006
Weight of kidney of high dose groups	0.545 ± **0**.132	0.838 ± 0.036	0.001

Table 2. Comparison of weight of kidney of high dose and low dose experimental group of rat.

Parameter (Mean ± SD)	Experimental high dose groups, *n=15*	Experimental low dose groups, *n=15*	P –value
Weight of kidney	0.838 ± 0.036	0.647 ± 0.126	0.001

Table 3. Diameter of Proximal and Distal Convoluted Tubule (PCT and DCT) of rat.

Parameter (Mean ± SD)	Control (n=15)	Experimental (n=15)	P- value
Diameter of PCT of low dose groups	36.5 ± 6.72	39.6 ± 6.80	0.001
Diameter of DCT of low dose groups	**23. 6** ± 6.30	30.0 ± 8.16	0.001
Diameter of PCT of high dose groups	40.1 ± 8.70	44.9 ± 7.72	0.001
Diameter of DCT of high dose groups	26.2 ± 6.32	32.6 ± 9.39	0.001

Table 4. Comparison of change in Diameter of PCT and DCT of High dose and Low dose Experimental group of rat, *n=60*

Parameter (Mean ± SD)	Experimental high dose groups (n= 15)	Experimental Low dose groups (n= 15)	P value
Diameter of PCT	44.9 ± 7.72	39.6 ± 6.80	0.001
Diameter of DCT	32.6 ± 9.39	30.0 ± 8.16	0.035

Table 5. Diameter of Renal corpuscle, Glomerulus and Bowman's Space of rats, *n= 60*

Parameter (Mean ± SD)	Control (n=15)	Experimental (n=15)	P- value
Diameter of renal corpuscle of low dose group	90 ± 5.12	112.3 ± 10.43	0.001
Diameter of glomerulus of low dose group	79.5 ± 4.79	90.8 ± 11.52	0.001
Diameter of Bowman's space of high group	10.5 ± 2.19	21.5 ± 5	0.001
Diameter of Renal corpuscle of high group	102.85 ± 11.55	128.20 ± 15.53	0.001
Diameter of Glomerulus of high group	89.5 ± 11.38	98.00 ± 14.63	0.001
Diameter of Bowman's space of high group	13.35 ± 5.86	30.8 ± 10.32	0.001

Table 6. Comparison of Renal Corpuscle, Glomerulus and Bowman's space of High dose and Low dose Experimental group of rat, *n= 60*

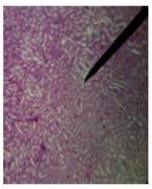
Parameter (Mean ± SD)	Experimental high dose groups (n=15)	Experimental low dose groups (n=15)	P- value
Diameter of renal corpuscle	128.2 ± 15.53	112.3 ± 10.40	1.60
Diameter of glomerulus	98 ± **1**4.63	90.8 ± 11.52	0.75
Diameter of Bowman's space	30.8 ± 10.32	21.5 ± 5	0.91

Qualitative Changes

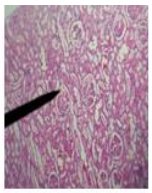
Hemorrhagic areas were seen in medullary areas of kidney of experimental low dose and high dose experimental group rat as shown in (**photomicrograph 2 and 6)**; however no hemorrhagic areas were observed in case of control groups as shown in (**photomicrograph 1 and 5**). Deformity in the proximal and distal convoluted tubule, atrophy of tubular epithelium, and lymphocytic infiltration in Bowman's space was noted in experimental high and low dose group as shown in (**photomicrograph 4 and 8 respectively**) while such changes were not appreciated in case of control groups rat as shown in (**photomicrograph 3 and 7 respectively**).

Photomicrograph 1: Medulla of control group rat (H &E ×100).

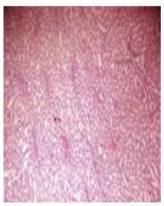
Photomicrograph 2: Medulla of experimental low dose group rat (H &E ×100). Arrow showing hemorrhagic spot



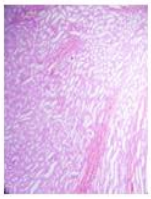
Photomicrograph 3: Showing cortex control group (H &E ×100). Arrow showing hemorrhagic spot



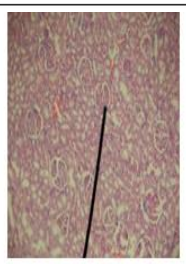
Photomicrograph 4: Showing cortex of experimental low dose group (H &E × 100). Arrow showing increased Bowman's space with lymphocytic infiltration.



Photomicrograph 5: Medulla of control group rat (H &E × 100). Arrow showing hemorrhagic spot



Photomicrograph 6: Medulla of experimental high dose group rat (H &E × 100). Arrow showing hemorrhagic spot



Photomicrograph 7: Showing the cortex of Control group rat (H &E × 100). Arrow showing Bowman's space

Photomicrograph 8: Cortex of high dose Experimental group rat. (H & E × 100) Red arrow showing hemorrhage and black arrow showing lymphocytic infiltration.

Discussion

Hemorrhage was seen in the medulla of experimental low dose and high dose group rat as shown in photomicrograph (2 and 6) respectively. Hemorrhage was also seen in the cortex of experimental high dose group rat as shown in photomicrograph (8).

Dilatation of renal tubule was observed in both experimental high dose and low dose group rat. Similar changes were observed in a study conducted by **Ghonim** in which changes like lymphocytic infiltration in Bowman's space, local hemorrhage and cystic dilation of renal tubules of progesterone treated animals are mentioned.[17] Lymphocytic aggregation in Bowman's space can be because of mesangial cells proliferation and compression of capillary. Hemorrhage seen can be because of vascular injury and due to the presence of tumors associated with DMPA injection while tubular dilatation can be because of tubular stasis, excessive renal hemodynamic changes and electrolyte and water loss after DMPA administration.

Atrophy of tubular epithelium was observed in the experimental group rats. Similar changes were observed in a study conducted by **Ghali** in which changes like increased diameter of renal tubules, congestion of renal corpuscle and atrophy of tubular epithelium in DMPA treated rat were seen.[18] This can be because of the disturbance in the cell growth and differentiation due to the effect of steroid hormone which can also affects the cell membrane permeability and integrity causing disturbance in equilibrium between intra and extracellular fluid.

Mean difference in change in the diameter of renal corpuscle, glomerulus and Bowman's space of experimental low dose was higher than control. Increased diameter of renal corpuscle, glomerulus and Bowman's space of low dose control and experimental group rat is shown in photomicrograph (3 and 4) respectively.

Degeneration of some renal corpuscle was observed in cortex of experimental group rats. Similar changes were observed in a study conducted by **AL-Rawi and MM** in which histological changes like degeneration of some renal corpuscles with progressive degeneration of their nuclei and necrosis, perivascular and interstitial lymphocytic infiltrations were seen.[19]This can be due to the renal injury caused by toxic effect of DMPA.

This study revealed dose and time dependent weight gain in experimental rats, similar change was seen in a study conducted by **Bakry et al.** in which significant dose dependent increase in weight of body and kidneys of all DMPA treated group rats was reported.[20] This can be due to direct effect of Depot –Medroxyprogesterone acetate (DMPA) injection on adipose tissue which lead to fat deposition causing weight gain. Similar result was revealed in a study conducted by **Rudel and Kincl** where rats were treated with 50 and 200 mg progesterone daily for 7 days and they revealed higher weight gain in the experimental high dose group rat than the experimental low dose group revealing proportional relation between the weight gain and dose of progesterone.[21] This can be because of the increase in serum lipids after progesterone administration. This study conclude that DMPA can cause both quantitative and qualitative changes in the histology of kidney depending on the dose and duration of drug.

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Conflict of Interest

No conflict of interest among the authors.

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