ejpmr, 2017,4(3), 251-256.

SJIF Impact Factor 4.161

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

EFFECTS OF DEPOT-MEDROXYPROGESTERONE ACETATE ON WHITE ALBINO RAT'S LIVER: A HISTOLOGICAL STUDY

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Article Received on 08/01/2017 **Article Revised on 29/01/2017** **Article Accepted on 19/02/2017**

ABSTRACT

Depot- medroxyprogesterone acetate (DMPA), the progesterone only contraceptive is widely used and has provided women a way for safe fertility as well as emancipating women from unwanted conception. DMPA is an aqueous suspension of medroxyprogesterone acetate (MPA), a synthetic analog of 17α-hydroxyprogesterone and variant of the human hormone progesterone. Like other drugs DMPA has its own merits and demerits. Despites of its huge acceptance it's not free of controversy and contraindication. Even though, it is mainly confined to reproductive tract. However, its effects are also seen in kidney, intestine, hypothalamus and liver. Present study aimed to see the histopathology of the DMPA treated rat's liver for assessing the hepatotoxicity. The histological architecture of liver of the DMPA treated rat was found to be distorted. Hemorrhage, congestion of the central vein and dilatation of the hepatic sinusoids were remarkable in the DMPA treated rat. The histological distortions were dose and duration dependent**.** This concludes that long term use of DMPA may be toxic to liver. So DMPA use should be under the supervision of the medical personnel**.**

KEYWORDS: DMPA, congestion, sinusoids, accentuation, hemorrhage.

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 (DMPA) and norethisterone enantate.[3]

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, 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 menstrual 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 has been proven to be relatively safe for lactating women and is free from the adverse effect of estrogen 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.[13] It can provide a suitable alternative method of contraception for women who are unable to tolerate oral methods e.g. women with inflammatory bowel disease (IBD) or malabsorption problems.[14]

Endogenous natural progestins are essential for the initiation and maintenance of pregnancy but exogenous

progestins and their derivatives induce adverse effect like delay in the return of fertility.[15] Other side effects of DMPA are 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).[16]

Drug such as cyproterone acetate (CPA) having progestational activity was found to be detrimental to liver inducing tumor growth.[17] This drug was also responsible for micronucleus in rat liver cells, chromosomal aberrations in V79 cells and also sister chromatoid exchanges in human peripheral blood lymphocyte in vitro.[18]

Drug having progestational activity caused abnormal liver function due to impairment in biochemical parameters like alanine transaminase (ALT), asparate transaminase (AST), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH).[19] Since liver is the decisive organ for metabolism of steroids hormone and accounts for approximately two-thirds of progesterone metabolism. But only few studies have been carried out to reveal whether DMPA cause any alteration in liver architecture, indeed liver function or not. Therefore, the present study was conducted to observe the changes in liver architecture and to interpolate effects of DMPA in liver of white albino rats.

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 in sufficient amount. They were maintained in a well ventilated room at controlled ambient temperature (25°c) with 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**. Each 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 12 weeks respectively. The control groups were given 0.25 ml and 0.5 ml of normal saline intramuscularly for 8 and 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.[20]

Drug to be given for rat = 0.018 × Human dose

Experimental Design and Treatment Regimen:

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

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

Low dose and high dose animal were sacrificed one day after the completion of experimental period. The rats were anesthetized with Ether soaked in cotton and liver were fixed by In Vivo Perfusion method. After completion of perfusion, the liver was isolated from the body with help of scalpel and forceps and post fixed for 24 hours with Bouin's Fluid. Thus obtained liver were cut into pieces of 3 mm to fix in neutral buffered formalin for 7 days and processed for making paraffin blocks. The blocks were trimmed, sectioned at 5 µm thickness and stained by routine H&E (Hematoxylilin and Eosin) staining. All sections were examined under light microscope.

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

Study design: case and control experimental study

RESULTS

Qualitative changes

Histology of liver of DMPA treated rats, both high and low dose group was altered as compared to control group rats. These alterations were more appreciable in high dose group rat than low dose group rat. Accentuation of hepatic lobule, congestion of central vein and hemorrhage in the liver parenchyma and dilation of hepatic sinusoids were observed in high dose group rat as shown in fig.1, fig. 2, and fig. 3 respectively.

While, hemorrhage and congestion of central vein and dilation of hepatic sinusoid was appreciable in low dose group but accentuation of hepatic lobule was not appreciable as shown in fig. 5 and fig. 6 respectively.

High dose control group rat's liver histology is shown in Fig. 4 and low dose control group rat's liver histology is shown in Fig. 7.

High dose group rat

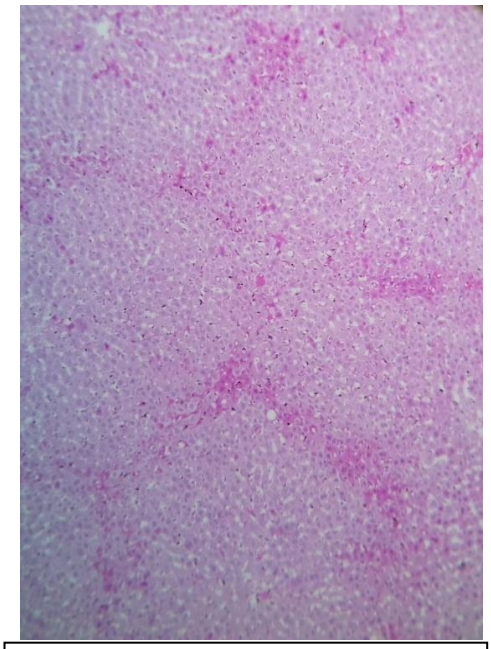

Figure 1: Histology of high dose group rat's liver showing accentuation of hepatic lobule (H &E × 10)

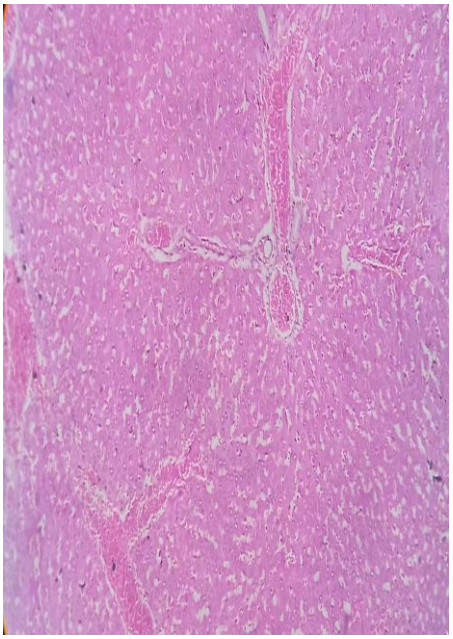

Figure 2: Histology of high dose group rat's liver showing hemorrhage and central vein congestion (H &E × 10)

Low dose group rat

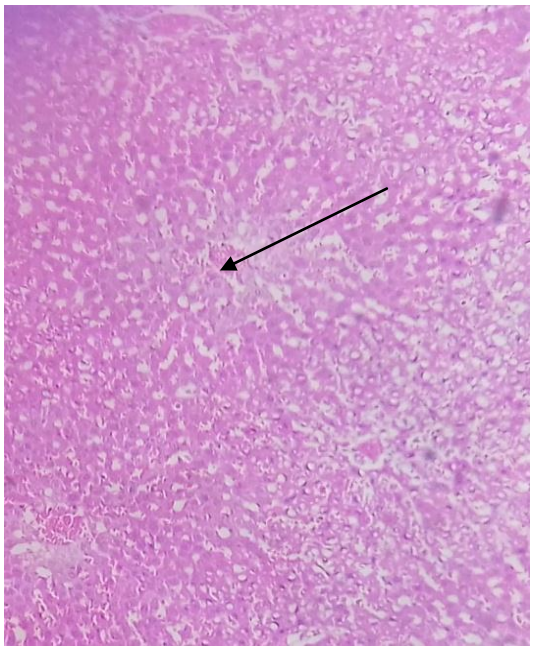

Figure 3: Histology of the high dose group rat's liver showing sinusoidal dilatation. (H &E × 10)

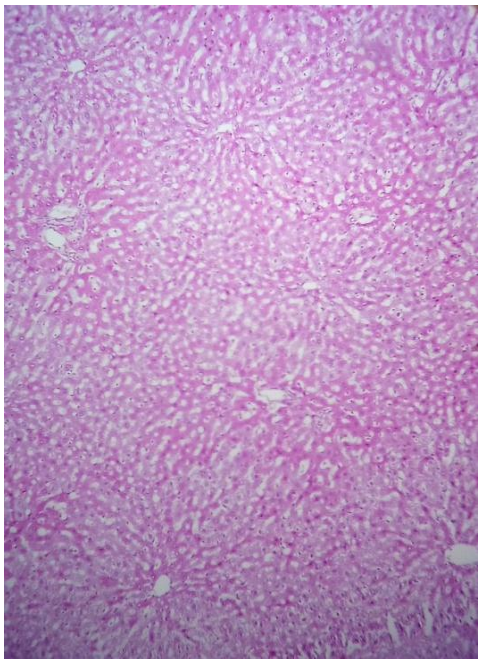

Figure 4: Histology of control high dose group rat's liver (H &E × 10)

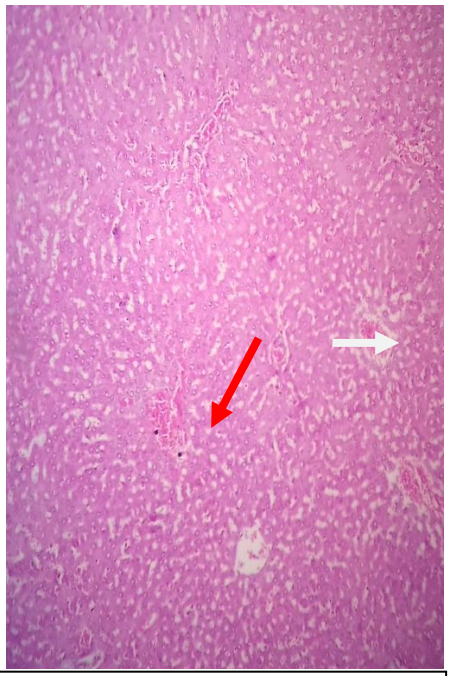

Figure 5: Histology of the low dose group rat's liver showing central vein congestion and hemorrhage (red arrow). (H &E × 10)

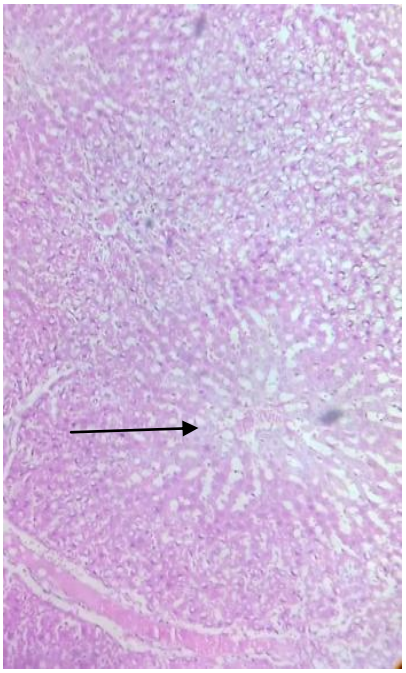

Figure 6: Histology of the low dose group rat's liver showing sinusoidal dilatation. (H &E × 10)

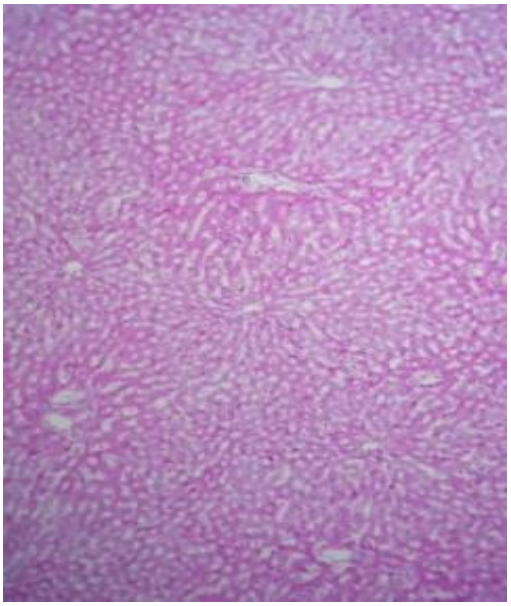

Figure 7: Histology of the control low dose group rat's liver (H &E × 10)

DISCUSSION

Depo-Provera is the most widely used long-term reversible contraceptive throughout the world. Depot-medroxyprogesterone acetate (DMPA) is a drug very similar to progesterone; a hormone normally produced by the ovaries every month as part of the menstrual cycle and has a weak androgenic property which prevents pregnancy for up to 3 months with each injection.[21] With the increasing use of the DMPA, the study about its merits and demerits has become more inevitable. Thus the present study was done to observe the effects of DMPA on the liver of white albino rat.

DMPA induced different histophathological lesions which were found to be dose and time dependent. The most remarkable effect of DMPA was the disturbance of the liver architecture. The liver sections of the control

rats exhibited the classical histological appearance as shown in fig. 4 and 7.

Accentuation of the hepatic lobule hemorrhage and central vein congestion, sinusoidal dilatation were observed in high dose group rats as shown in fig. 1, fig. 2 and fig.3 respectively. The findings of this study were concurring with the findings of the **Boseila et al** who reported dilatation of hepatic sinusoids, cellular infiltration and congestion of hepatic blood vessels following treatment with progesterone.[22] Hemorrhage seen in this section can be because of vascular injury and due to the presence of tumors associated with DMPA injection. The sinusoidal dilation and congestion can be due to the obstruction of the venous out flow.

In this study some of the histological sections were observed with vacuolation and fat infiltration in the hepatocytes. These observations were in accordance with a study conducted by **Attia et al** which revealed vacuolation, fatty infiltration and necrosis in liver tissues that was attributed to metabolizing of sex hormones in the hepatocytes which may leads to increase in the smooth endoplasmic reticulum and swollen mitochondria and cellular granularity.[23] Similar disturbance in the histological architecture of DMPA treated rat's liver were reported in another study conducted by the **Bakry. S and Abu-Shaeir**. **W**.[24] Cytoplasmic vacuolization, nuclear pyknosis, dilated blood sinusoids, congested blood vessel, hepatic cells enlargement with fatty infiltrates and cytoplasmic vacuoles were reported.

These histo-pathological lesion induced by the DMPA can be ascribed for its toxic effect.

CONCLUSION

The drug is mainly confined to the reproductive tract but their receptors (progesterone receptor, PR) are also found in other parts of body like breast, CNS and pituitary. Membrane progesterone receptor (mPR) is also found in neural, kidney and intestinal tissues. Many effects of progesterone are mediated by this progesterone receptor (PR). Although the progesterone receptor is not present in adult liver its effects were seen. The present study revealed that in high dose group rats there was significant hemorrhage, accentuation of hepatic lobule, congestion of central vein and dilatation of the hepatic sinusoids. The severity of hemorrhage was observed more in high dose group than in low dose group rat. This finding concludes that the effect of DMPA is both time and dose dependant. There is need of more research regarding the enormous use of contraceptives and their long term effects. Since the unmonitored use of steroidal contraceptive is mounting in the developing countries, the women are more exposed for its adverse effects. This study may contribute for the data banking in food and drug administration stand points and this can also provide reliable basis for instituting appropriate strategies regarding the use of DMPA.

ACKNOWLEDGEMENT

We would like to express grateful thanks to BP Koirala Institute of Health Sciences, Nepal for providing research facilities and we are also thankful to all staffs, research laboratory and animal house staff for their proper supports during study.

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