

EFFECT OF HORMON REPLACEMENT THERAPY ON TOTAL BODY WEIGHT, GROSS APPEARANCE, RELATIVE ORGAN WEIGHT AND VOLUME OF LIVER IN OVARIECTOMIZED RAT MODEL

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ABSTRACT

Objective: To evaluate the effects of hormone replacement on gross appearance, total body weight and volume of liver in ovariectomized rats.

Study Design: Laboratory-based experimental study.

Place and Duration of Study: Department of Anatomy, Army Medical College, Rawalpindi, in collaboration with National Institute of Health, Islamabad, from Mar to Jun 2019.

Methodology: Forty, female, healthy, non-pregnant, Sprague dawley rats, 5-6 months of age, weighing 250 ± 10 g, were randomly divided into 4 equal groups (A, B, C and D). Group A served as control and group B was sham operated. Group C i.e. ovariectomized group underwent ovariectomy but was kept untreated. Group D underwent ovariectomy and was treated with injection estradiol (Inj Gravibinan, 2ml, Medipharm. Mfg. Co, Ltd) 2.5 mg/kg via subcutaneous route once weekly for 60 days. All animals were sacrificed after the last injection of estradiol as hormone replacement therapy (HRT) to group D. Gross parameters of liver were observed. Results were analyzed using SPSS version 22. The p -value of ≤ 0.05 was considered as significant.

Results: Ovariectomized (OVX) group showed marked change in colour and consistency of liver, increased total body weight and volume of liver. While these changes started reverting back towards normality in treated group.

Conclusions: Injection of hormone replacement therapy improved the gross parameters of liver in treated group, 12 weeks after surgery as compared to untreated group.

Keywords: Hormone replacement therapy, Gross appearance, Liver, Ovariectomy, Relative weight, Volume.

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INTRODUCTION

Benign neoplastic cystic tumors are the most common in young women and have the potential to spread beyond the ovary. A study conducted in 2016 proved statistically significantly increased risk of serous ovarian cancer among women. The mainstay of the management for these tumors remains surgery with removal of affected ovaries¹. In short, Ovarian cysts, ovarian torsion, tuboovarian abscess, endometriosis and endometrial carcinoma are the causes in which ovariectomy becomes the treatment of choice in young females. Primary ovarian insufficiency (POI) is a rare but important cause of sex steroid deficiency in young females². Such disorders may lead to

estrogen depletion³ which is usually associated with an increase in body weight and body fat accumulation leading to an increased risk of metabolic syndrome, nonalcoholic fatty liver disease (NAFLD), and heart disease⁴. Recent studies have demonstrated that mice with estrogen receptor alpha (ER α) mutations, ovariectomized (ovariectomized) rodents, mice with global ER α knock-out, and mice lacking aromatase may undergo loss of estrogen signaling, thereby leading to an increase in liver fat. Estrogen deficiency leads to redistribution of body fat and accumulation of visceral fat, which may cause nonalcoholic fatty liver disease development and progression⁵.

Estrogen prevents liver fat accumulation and stimulates fat oxidation, thereby preventing insulin resistance⁶. It also plays an important role in controlling biological activities. The effects of estrogen are mediated through receptor alpha (α)

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and beta (β) placed on many extragonadal sites e.g. liver, heart, muscle, bone and brain⁷. Estrogens induce hepatocyte proliferation. These multiple effects are mediated mostly by ER α . Moreover, estradiol (E2) signaling in the liver is a genomic reaction which indicates that ER α and/or ER β are targets for E2 signaling in the liver⁸. Estradiol shows potent anti-proliferative and anti-inflammatory properties in nonalcoholic fatty liver disease⁹.

The studies have focused the histological effect of ovariectomy on salivary glands. Moreover they have shown that HRT reduces degenerative changes in oral epithelium secondary to ovariectomy¹⁰. Many studies have been published in the recent years showing role of HRT in ameliorating histological changes in thyroid. Effects of ovariectomy have been studied in liver lipid metabolism and gene expression in rat liver⁸⁻¹⁰. Yet we spotted a scarce literature commenting on gross parameters of marked tissue in the absence of this influential hormone. Speculating this, we planned a study whose purpose was to observe the outcome of hormonal therapy on colour, consistency, volume and weight of liver in ovariectomized rat model however role of extragonadal estrogen would also be accessed in untreated experimental rats. If HRT is found to have ameliorating effect, it may become a useful guide for ovariectomized patients to avoid fatty changes in liver which if left untreated, may progress to Nonalcoholic steatohepatitis (NASH).

METHODOLOGY

This research was carried out in the department of Anatomy, Army Medical College, Rawalpindi, in collaboration with National Institute of Health (NIH), Islamabad, from March to May, 2019 after approval by Ethical Review Committee of the Army Medical College, Rawalpindi and National University of Medical Sciences, Islamabad.

It was a laboratory based experimental study and non-probability consecutive sampling technique was used. Forty, healthy, female, 5-6 months old, "Sprague Dawley" rats; Weighing 250 ± 10

gm were selected. Animals with any visible pathology in the body were excluded from the study. They were kept in separate cages at controlled room temperature of $20 \pm 2^\circ\text{C}$ and 12-hour light and dark cycles were maintained. They were fed on standard laboratory rat chow and water ad libitum.

Rats were randomly divided into four groups A, B, C and D having 10 animals in each group. Rats in group A served as control which only received standard diet and water. While group B, C and D served as experimental groups. Group B was sham operated, while group C and D underwent ovariectomy. To begin with experiment all animals belonging to experimental groups were anesthetized by giving intramuscular injection of a mixture of xylazine-ketamine hydrochloride (xylazine: 5mg/kg and ketamine: 35mg/kg). Anesthetized animal was placed on its dorsal surface. The fur on the rat abdomen was removed with a shaver. The area of surgery was cleaned with ethanol. A small transverse skin incision was made with surgical scalpel blade no. 11 on the middle part of the abdomen slightly towards right, just near to the right nipple of the rat. After accessing peritoneal cavity, the adipose tissue was pulled away until the right uterine tube and the ovary was identified. After identifying the ovary and uterine horn, a braided silk suture (Ethicon mersilk sutures-3/0, Johnson & Johnson Ltd) was applied around the area of the distal uterine horns, that was sectioned thereafter to remove the ovaries. The ovary and associated fat was located and exteriorized by gentle retraction. The procedure was repeated for the left ovary through the same incision slightly extending it to the left. The periovarian fat with the ovary was pulled away from the incision site gently to prevent detachment of a small piece of ovary, which may fall into the abdominal cavity where it may be reimplanted and carry on its normal function. The uterine horn was then returned to the peritoneal cavity after the removal of ovaries. The wound was closed in two layers (muscle and skin) using sterile sutures.

The sham-operated group underwent a similar surgical procedure, exposing the ovaries but placing them again in the same position. Each rat of experimental group received an intramuscular injection of 0.1 ml Penicillin G procaine (300,000 units, Shanxi shuguang Pharmaceutical Co Ltd, China) daily for one week¹³. After one day of last antibiotic injection Group B and C were given subcutaneous injection of sesame oil (0.1 ml/day) once daily, while group D was treated with subcutaneous injection of estradiol 2.5mg/kg/day (inj gravibinan) dissolved in sesame oil (0.1ml/day) once daily for 60 days.

After 12 week rats were weighed to calculate weight gain. The rats were then sacrificed by

tative parameters e.g body weight gain, volume and relative weight of organ were expressed as mean \pm SD. Significant difference was determined by using oneway analysis of variance (ANOVA) followed by post Hoc Tukey test. Qualitative variables such as colour and consistency of liver were expressed as frequency and percentages. Chi square test was used for intergroup comparison among these variables. The p -value ≤ 0.05 was considered significant.

RESULTS

All the animals stayed alive healthy and active till the end of study. Liver specimens belonging to control group A were reddish brown in colour and soft to firm in consistency

Table-I: Comparison of frequency and percentages of qualitative parameters between control group A and experimental groups C and D.

Parameter	Findings	Groups of animals				p -value		
		A	B	C	D	A Vs C	A vs D	C Vs D
Colour (appearance)	Reddish brown	10 (100%)	10 (100%)	4 (40%)	7 (70%)	0.011*	0.211	0.239
	Pale brown	-	-	3 (30%)	3 (30%)			
	Dark brown	-	-	3 (30%)	-			
Consistency	Soft to firm	10 (100%)	10 (100%)	4 (40%)	7 (70%)	0.003*	0.211	0.113
	Friable & greasy	-	-	3 (30%)	3 (30%)			
	Nodular	-	-	3 (30%)	-			

Table-II: Comparison of mean values of quantitative parameters between control group A and experimental groups C and D.

Parameters	Mean \pm SD				p -value		
	Group A	Group B	Group C	Group D	A vs C	A vs D	C vs D
Weight gain	2.5 \pm 0.7	2.5 \pm 0.7	4.1 \pm 0.9	3.0 \pm 0.7	0.001	0.6	0.01
RTBWI	2.8 \pm 0.2	2.8 \pm 0.2	3.1 \pm 0.01	2.6 \pm 0.2	0.001	0.02	0.001
Volume of liver	708.5 \pm 41.9	718 \pm 104.3	817 \pm 38.9	765.5 \pm 102.8	0.01	0.3	0.03

overdose of anesthetic agent chloroform. The animals were fixed to dissection board and a longitudinal midline incision was made below the ribcage to cut through skin and muscles. Gross analysis of liver based on its colour, consistency and appearance was done. Weight was recorded in gm using digital precision balance and volume was measured in mm³ by formalin displacement method¹¹⁻¹⁴. Relative liver weight (RTBWI) was calculated using following formula

$$\text{RTBWI} = \frac{\text{Weight of Organ in gm}}{\text{Weight of body in gm}} \times 100$$

Data was analyzed by using SPSS (Statistical Package for Social Sciences) version 21. Quanti-

which was completely normal. Findings of group B were consistent with those of group A. However, in group C 40% specimen showed normal colour, 30% were pale brown and 30% were dark brown. Moreover, in treated group D, 70% animals showed normal colour while 30% had a mild change in colour. Regarding consistency of liver in group C, 40% were normal, 30% were friable and greasy and rest 30% were nodular. While group D showed normal liver except 30% which were nodular.

On intergroup comparison significant change in colour and consistency were seen in experimental group C when compared with control

group A (p -value=0.011 & 0.003 respectively). However, in experimental group D these changes were insignificant when compared with control group A and experimental group C (p -values

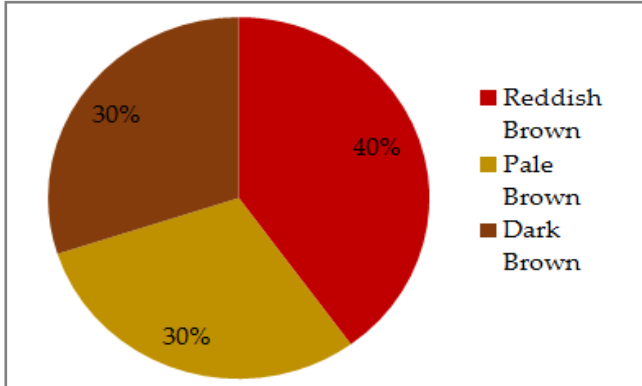


Figure-1: Pie chart showing percentages regarding colour of liver in experimental group C.

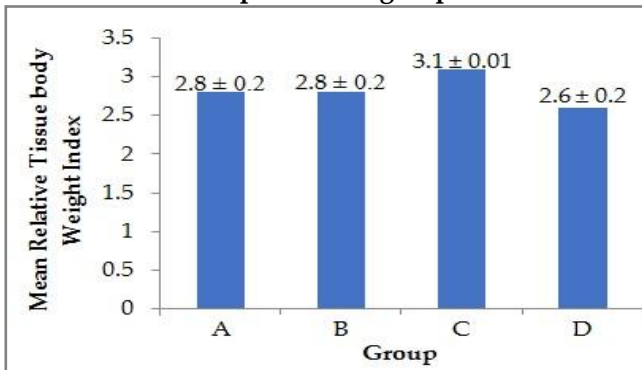


Figure-2: Cluster bar chart showing relative liver weight along with standard deviation among control and experimental groups.

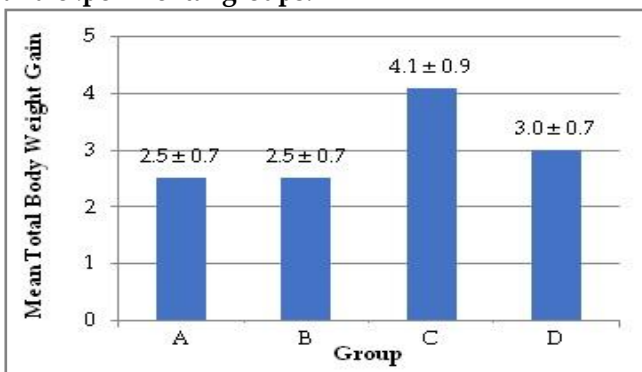


Figure-3: Cluster Bar chart showing total body weight gain along with standard deviation among control and experimental group.

<0.05).

Regarding mean total body weight gain, relative liver weight and volume there was no

difference in findings between control group A and experimental group B. The mean total weight gain in experimental group C was extremely significant when compared with group A (p -value 0.001) and group D (p -value=0.01). However, this parameter was statistically insignificant in experimental group D when compared with control group A (p -value 0.6).

Relative weight of liver increased significantly in intergroup comparison of A and C and D (p -value<0.05). Volume of liver showed a significant increase when experimental group C was compared with control group A (p -value 0.01) and D (p -value 0.03), but the change was insignificant when control group A was compared with treated group D (p -value<0.05).

DISCUSSION

The younger the woman at time of oophorectomy, the greater the risk of deleterious effects. The reason being estrogen deficiency in the body, which causes accelerated aging in many organ system¹³⁻¹⁵. That’s why young rats were ovariectomized to observe alteration in gross appearance of liver in the absence of gonadal estrogen, as it is one of the parameters to assess progression towards liver disease. Change in total body weight, relative organ weight and volume of liver were also accessed. Subcutaneous estradiol was given as a treatment to show reversal of these change in experimental group D.

There was significant weight gain when control group A was compared with experimental group C (p -value 0.001) which was more remarkable than a study conducted by Neto and his colleagues who demonstrated notable weight gain (p -value<0.05) in ovariectomized animals¹⁶. Interestingly, replacement of estrogen reduced body weight of ovariectomized rats belonging to group D (p -value<0.01) when compared with group C. Lower weight gain in treated group D than those untreated rats in group C indicates that low estrogen levels after ovariectomy is responsible for the body weight gain. These results are consistent with a study conducted in 2017 in which ovariectomized receiving estrogen imp-

lants in medial amygdala showed significant decrease in body weight (p -value <0.05) as compared to control group¹⁷.

Regarding gross parameters, all the livers of control and sham group were reddish brown in colour and soft to firm in consistency. Contrary to this in experimental group C only 40% livers were normal whilst 30% liver were pale brown (yellow) & friable (greasy) and rest were dark brown and nodular (A vs C, p -value 0.01) This is in accordance with a study according to which steatotic liver has significant chances of having pale-yellow appearance, greasy consistency and multinodular focal spots¹⁸. The colour is largely caused by retained carotenes and the consistency owing to fat accumulation. However, when estradiol was given to experimental group D the colour and consistency of liver start reverting back towards normal which gives evidence of beneficial role of estrogen on liver architecture.

Only a few studies have demonstrated the effect of hypoestrogenism on relative liver weight. When this parameter was calculated OVX group showed a highly significant increase (p -value 0.001) in group C. These results are in agreement with two manuscripts. One was conducted by Luo and his colleagues who were in favor of significant increase (p -value <0.05) in relative liver weight in OVX rats¹⁹, while other study established the same fact with high significance (p -value <0.01)²⁰. Administration of estradiol in rats belonging to group D showed a significant decrease in liver weight when compared with group C (p -value 0.001) which is in accordance to a recent study in which OVX rats showed a significant decrease in relative liver weight (p -value <0.01) after estradiol treatment emphasizing the fact that HRT ameliorates OVX-induced fatty liver⁹.

This study proved that ovariectomy significantly increased the liver volume in experimental group C when compared with control group A (p -value 0.01) which supports the study conducted by Bian and his colleagues who proved that liver volume is increased in nonalcoholic

fatty liver disease (p -value <0.001)²¹. Interestingly, when treatment was given, volume significantly reduced in experimental group D (p -value 0.03) in comparison with untreated group reinforcing the curative effects of estrogen therapy.

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Disclosure

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CONCLUSION

Hormone replacement therapy has a significant curative effect on altered colour and consistency of liver. It controls total body weight and liver volume. Early treatment may prevent progression to high grades of liver disease which may require interventions that severely effect patient's quality of life.

CONFLICT OF INTEREST

This study has no conflict of interest to be declared by any author.

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