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ORIGINAL ARTICLE

Histological Effects of Cotton seed Oil on Ovaries and RTWI among Female Albino Rats: RCT

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ABSTRACT

Background: Concerns over the rising infertility rate among humans is now becoming a global health issue.
Aim: To observe the histological changes in ovaries while measuring relative tissue weight indices of all ovaries among different groups receiving cottonseed oil.
Study Design: Randomized controlled trial.
Methodology: Twenty four adult female albino rats (12 weeks of age) were divided into 04 groups. Group-1 (Control) rats were given orally distilled water @ 400mg (equivalent to 400µl)/kg body weight per day. Group-2, Group-3 & Group-4 rats were given cottonseed oil (treatment groups) @ 400mg (equivalent to 432µl)/kg body weight per day, respectively, for a period of 30 days.
Results: Cottonseed oil significantly reduced fertility in terms of follicles and ova in all treatment groups than control having non-significant differences among means of different groups.
Conclusion: This study concluded that the crude cottonseed oil significantly affected female rat fertility in terms of reduced number of follicles and ova as shown histologically**.** However, it did not affect relative tissue weight indices among different groups. Hence, its use in edible products may result in increased women infertility.
Keywords: Cottonseed Oil, Relative Tissue Weight Indices and Number of Follicles.

INTRODUCTION

Increasing infertility rate among humans is now becoming a global health issue including developing countries[1,2]. Due to exposure to environmental contaminants, fertility has been at risk thus increasing the concerns[3]. Anthropogenic processes expose humans to thousands of chemicals. Several solvents, heavy metals, pesticides and rest of the persistent organic pollutants are under observation as environmental factors. In our environment, pesticides are omnipresent contaminants[4,5]. It has been estimated that almost one third of female infertility was due to ovulation problems. Hormonal imbalance (LH and FSH), brain injury (hypothalamus or pituitary gland) and pituitary tumors are the contributing factors[6]. Several factors like lifestyle, advanced maternal age, occupational risk factors and exposure to chemicals present in environment and diet affect woman's ability to get pregnant as revealed by literature review.[7]

According to an estimate, infertility hits every 7th couples in the UK. Factors like better awareness about contraception and increasing workload due to changed role of women played a significant role besides other factors[8]. Although around 26% of infertile couples have unexplained infertility. Genetic transfer of traits in transgenic plants is another area related to environmental concerns. Although, biotechnology has developed insect resistant crop varieties, including cotton, but at a cost of human health[9].

The only genetically modified crop approved for commercialization and grown in Pakistan is Bt cotton. Since edible cottonseed oil is being obtained from both conventional and biotech cottonseeds therefore present study was planned to observe the histological changes in

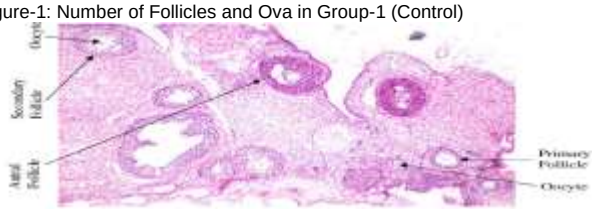
METHODOLOGY

Twenty four adult female albino rats (12 weeks of age) were divided into 04 groups. The animals were kept at 22±2°C at a humidity level of 55±10% with a 12:12 h light–dark cycle following the guidelines established by the Ethical and Practical Principles of the Use of Laboratory Animals and University of Health Sciences, Lahore[10]. Group-1 (Control) rats were given orally distilled water @ 400mg (equivalent to 400µl)/kg body weight per day. Group-2, Group-3 & Group-4 rats were given cottonseed oil obtained from insecticide free non-GM crop, cottonseed oil obtained from insecticide sprayed non-GM crop, cottonseed oil obtained from insecticide free GM (Bt) crop @ 400mg (equivalent to 432µl)/kg body weight per day, respectively, for a period of 30 days[11]. Right and left preserved ovaries of each animal were taken and put in tissue cassettes properly labeled. The tissue was processed in the automatic tissue processor for 18 hours. In processing, first step is dehydration in which the tissues are dehydrated by passing through ascending grades of alcohol. The dehydration was done to remove aqueous fixative fluid from the tissues and replace them with dehydrating fluid. Second step was clearing to completely replace the dehydrating agent with a fluid that is totally miscible with both dehydrating fluid and embedding medium. The hydrocarbon solvents such as xylene were used for this purpose, giving tissue a translucent appearance. Third step was impregnation in which clearing agents were replaced with embedding medium i.e. Paraffin wax. Data analyzed by SPSS 24.0v. One-way Analysis of Variance (ANOVA) and post hoc Tukey's Multiple Comparison Test were applied.

RESULTS

Number of Follicles: Ovarian follicles were counted using Olympus CX-21 microscope. All follicles whether containing an oocyte or not were considered in counting. All primordial, primary, secondary and antral follicles were counted under 10X and 40X lenses. Both left and right ovaries of each rat were used for counting.
Number of Ova: No of ova were counted using Olympus CX 21 microscope. The ova clearly visible in all follicles (primordial, primary, secondary and antral) were considered for counting, using 10x and 40x lenses. Both right and left ovaries of each rat were taken. All primordial, primary, secondary and antral follicles were counted as shown in figure-1.

Figure-1: Number of Follicles and Ova in Group-1 (Control)



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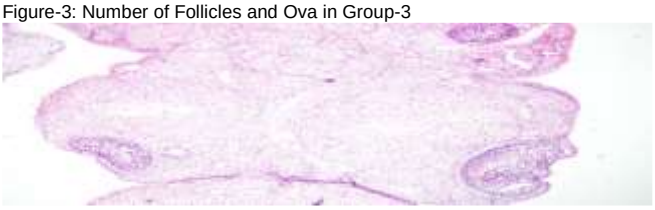
Reduced number of follicles and ova is visible in rats fed with cottonseed oil obtained from insecticide free non-GM crop as shown in figure-2.

Figure-2: Number of Follicles and Ova in Group-2

Reduced number of follicles and ova is visible in rats fed with cottonseed oil obtained from insecticide sprayed non-GM crop as shown in figure-3.

Figure-3: Number of Follicles and Ova in Group-3



Reduced number of follicles and ova is visible in rats fed with cottonseed oil obtained from insecticide free GM (Bt) crop as shown in figure-4.

Figure-4: Number of Follicles and Ova in Group-4

The results showed that relative tissue weight indices in control group and treatment groups have been 0.0243±0.00356, 0.0241±0.0057, 0.0193±0.0059 and 0.0165± 0.0106, respectively (Table-1). Analysis showed non-significant differences among means of different groups.

Table-1: Relative Tissue Weight Index (gm) in different Rat Groups

	Groups			
	G_1	G_2	G_3	G_4
Total	0.145783	0.144369	0.115899	0.099009
Means	0.024297	0.024062	0.019316	0.016501
SD	± 0.003558	± 0.005709	± 0.005942	± 0.010599
p-value	0.783			

DISCUSSION

Present study was carried out to compare the toxic effects of different cottonseed oil on fertility of female rats. Female Albino rats have short length of the estrous cycle that makes them ideal for study linked with reproductive cycle changes. Literature review has revealed that Wistar Albino rats were used in many studies in order to evaluate the toxic effects of GM food on various organ systems[12].

In present study, the relative tissue weight indices of the ovaries in all treatment groups showed insignificant differences in comparison to group (G1). Literature review revealed that cottonseed oil has gossypol content ranging from 0.05-0.54% hence, each dose in current study administered 0.2mg to 2.16mg gossypol/kg/day. Our results were in line with previous research that reported daily injections of 0.1 and 1.0 mg gossypol/kg/day had insignificant effect on ovarian weight[13].

Results in the present study regarding an estimation for the number of follicles and ova showed that treatment given had drastically reduced their numbers in the treated groups. Our results were in line with previous studies which suggested that there was a complete blockage of ovulation in animals receiving locally extracted “crude” cottonseed oil[14,15].

Limitations: Small sample size, financial constraints and limited resources were the limitations.

Conflict of interest: None

Funding: None

CONCLUSION

This study concluded that the crude cottonseed oil significantly affected female rat fertility in terms of reduced number of follicles and ova as shown histologically**.** However, it did not affect relative tissue weight indices among different groups. Hence, its use in edible products may result in increased women infertility.

Authors’ Contribution: MZ & GAK: Conception and design of work, **SA & AS:** Collecting and analyzing the data, **MS & TL:** Drafting the manuscript.

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