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Original Research Article

Extra Virgin Olive Oil Mitigated Uterine Dysfunctions in Female Wistar Rats Exposed to Benzene and Ethanol

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Abstract: ***Background*:** Exposure to benzene and ethanol is known to predispose to ovarian and uterine retardation, Extra virgin olive oil (EVOO) is an essential oil that is well-reputed for its outstanding nutraceutical and therapeutic properties. The present study is aimed at elucidating the pharmacological potentials of EVOO supplementation on ethanol/benzene-induced female reproductive dysfunctions in rats. ***Methodology*:** Forty-eight (48) adult Wistar rats weighing (110kg-130kg) were divided into 8 groups (n = 6). CTR= Control, EVOO= Extra Virgin Olive Oil, ETH= Ethanol, BEN= Benzene, ETH+BEN= Ethanol + Benzene, ETH+EVOO= Ethanol+ Extra Virgin Olive Oil, BEN+EVOO= Benzene+ Extra Virgin Olive Oil, ETH+BEN+EVOO= Ethanol+Benzene + Extra Virgin Olive Oil. They were administered Ethanol, Benzene and Extra Virgin Olive Oil according to the grouped tagging. At the end of the experiment, the rats were sacrificed while tissues were harvested, and processed for H & E stain and Masson Trichrome stain histopathological assessment. Progesterone and estradiol were measured using standard methods. Statistical analysis was done using Analysis of Variance (ANOVA) on GraphPad Prism 5.0 software. Significance was set at $p<0.05$. ***Results*:** Interestingly, EVOO supplementation significantly ($p<0.05$) increased body weight, estradiol, and progesterone levels, while also mitigating ovarian and uterine histoarchitectural integrity. ***Conclusion:*** EVOO may be considered when formulating a dietary regimen in the management of female reproductive dysfunctions associated with benzene and ethanol.

Keyword: Uterus, Extra Virgin Olive Oil, Benzene, Ethanol, Progesterone.

INTRODUCTION

Ethanol and benzene are oil-related petroleum products that are widely used as fuels, solvents and raw materials in the chemical industry [1]. However, studies on the Influence of oil-related environmental pollutants on female reproduction have shown that ethanol and benzene procure a degree of disruption to the luteal phase of the menstrual cycle sequencing [2]. And to the retardation of fetal growth during pregnancy [3]. It was reported that an increase in benzene concentration in the human ovarian follicular fluid can be associated with a downhill in oocyte and embryo production [4]. Studies on mice exposure to benzene have increased the incidence of ovarian granulosa cell tumors and ovarian benign mixed tumors [4]. Moreover, benzene has been reported to cause a variable increase in the incidence of aneuploidy in mature mouse oocytes [5]. Occupational exposure of women to various degree of these chemical compounds (benzene, ethylbenzene, toluene, and xylenes) have caused a reduction in the blood gonadotropin (follicle-stimulating hormone, luteinizing hormone) and prostaglandin level [2-6]. Although, high benzene level in ovarian follicular fluid was associated with increased follicle-stimulating hormone and decreased estradiol levels in blood plasma [4].

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Extra virgin olive oil (EVOO) is a well-reputed essential oil human diet with a positive effect on human health (Lombardo *et al.*, 2018). (EVOO) mainly constitutes triglycerides of about 98% and minor compounds ranging from 1–3% which are the major compositions responsible for its biological and bioactive properties. The high ratio of its lipid profile and have been linked to protective effects on coronary, autoimmune and inflammatory disorders but also as anti-thrombotic and regulators of blood pressure [7, 8]. Its associated properties reduce risk of several chronic illnesses, diabetes, hypertension, obesity and cardiovascular diseases [9]. Mounting evidences have revealed that (EVOO) processes antioxidant properties [10], and anti-inflammatory activity [11], with various bioactive compositions [12]. Its primary and secondary metabolites such as lipids, carbohydrates and phenolic compounds has compensated much more to its ranging bioactive properties in the action of antioxidant and anti-inflammatory effects. In our previous study, [13], we reported the potential therapeutic benefit associated with EVOO supplementation in benzene/ethanol induced hematotoxicity. However, given the evident cases associated of benzene and ethanol with effects on ovarian functions and its profile it is crucial to explore the effect of extra virgin olive oil on ovarian integrity. This study focuses on the bioactive properties of extra virgin olive oil on effects of benzene and ethanol on ovarian functions of female Wistar rats.

MATERIALS AND METHOD

A total of 48 female Wistar rats (weighing 150-200g) were used for the experiment. The animals were purchase and kept in a controlled environment on 12:12hr light/dark cycle. The animals were allowed to acclimatized for 14 days with unrestricted access to food and water *ad libitum* before the commencement of the study.

Animal Conditioning, Care and Management

The experimental animals were bred, kept and cared for in the experimental animal house, Babcock University, Ilishan-Remo, Ogun State according to the care and use of animals in research and teaching approved by the institute of laboratory animal research. The experimental animals were allowed to acclimatize for seven (7) days before the commencement of a pilot study with 2 female Wistar rats. On completion of the pilot study, research approval was gotten from the Babcock University Research Ethical Committee (BUHREC) with BUHREC NO 798/19.

Preparation and Procurement

75% ethanol was gotten from Sigma-Aldrich, 10mls of the 75% ethanol was mixed with 20mls of distilled water to give 30mls of 25% ethanol. It was administered at 2mls to the rats in group 2, 4, 5 and 7 orally individually or in combination respectively twice a week.

Benzene was purchased from University of Ibadan appropriate quantity was measured and kept in a dark glass reagent bottle. It was administered at 200mg/kg to the rats in group 3, 4, 6 and 7 orally individually or in combination respectively twice a week.

Extra virgin Olive oil was also purchased from Sigma-Aldrich and kept in a cool dry cabinet when not in use. It was administered at 2mls to the rats in group 5, 6, 7 and 8 orally individually or in combination respectively twice a week.

Animal Grouping and Administration

Administration started after one week of acclimatization and rats were administered via gastric gavage (oral route). GROUP 1: All animals were fed with animal feed and water until the day of animal sacrifice. GROUP 2: were given 25% ethanol (2ml) twice a week for 2 weeks. GROUP 3: were given benzene (200mg/kg body weight) twice a week for 2 weeks. GROUP 4: was given 25% ethanol (2ml) and benzene (200mg/kg body weight) simultaneously twice a week for 2 weeks. GROUP 5: were given 25% ethanol (2ml) twice a week for 2 weeks after which extra virgin olive (2ml) treatment started twice a week for the next two weeks. GROUP 6: were given benzene (200mg/kg body weight) twice a week for 2 weeks after which extra virgin olive (2ml) treatment started twice a week for the next two weeks. GROUP 7: were given 25% ethanol (2ml) and benzene (200mg/kg body weight) simultaneously twice a week for 2 weeks after which extra virgin olive oil (2ml) treatment started twice a week for the next two weeks. GROUP 8: were given extra virgin olive oil (2ml) twice a week for 2 weeks.

Experimental Design

	NO. OF RATS	TREATMENT SCHEDULE
Group 1	6	Animal feed and distilled water only
Group 2	6	Animal treatment with ethanol orally (2ml)
Group 3	6	Animal treatment with benzene orally (200mg/kg)
Group 4	6	Animal treatment with benzene (200mg/kg) + ethanol orally (2ml)
Group 5	6	Animal treatment with ethanol (2ml) + extra virgin olive oil orally (2ml)
Group 6	6	Animal treatment with benzene (200mg/kg) + extra virgin olive oil orally (2ml)
Group 7	6	Animal treatment with benzene (200mg/kg) + ethanol (2ml) + extra virgin olive oil orally (2ml)
Group 8	6	Animal treatment with extra virgin olive oil orally (2ml)

Measurement of Body Weight

The body weight of the rat was measured daily throughout the duration of the administration and stimulation with the use of a weighing balance. This was done in order to check the weight gain or loss in each group.

Mean Body Weight

The mean body weight was calculated as the initial body weight of the animals subtracted from the fin al body weight of the animal within the span of the experimental period i.e., [MBW=final body weight-initial body weight.

Animal Sacrifice

After 12 days of treatment, sacrifice was carried out through cervical dislocation. Blood was then collected from the left ventricle with needle and syringe and stored in heparinized bottles for hormonal (testosterone, follicle stimulating hormone and luteinizing hormone) assay which was then centrifuged at 3000RPM for 5 minutes to separate serum blood. The rats were then perfused using normal saline and 10%formaldehyde. The testes were then excised through lower abdominal incision using scapel and forceps and kept in sample bottles filled with 10% formaldehyde solution for histological analysis.

Hormonal Assay

Hormonal assessment was done by collecting 2ml of blood through the left ventricle of the heart into heparinized bottles and centrifuged at 3000 revolutions per minutes for 5 minutes using Gulfex medical and scientific centrifuge, England. The plasma was separated by decantation and analyzed for hormonal assay.

Histopathological Examination

The uterus was carefully excised, weighed, and fixed in 10% formo-saline solution. Thereafter, the tissues were embedded in paraffin wax, sectioned and stained using hematoxylin and eosin.

Statistical Analysis

The graph pad prism 5.0software was used for statistical analysis the raw data was converted to grouped and evaluated statistically using one-way analysis of variance (ANOVA). Student Newman-Klaus was to identify differences between each mean and a value of $p<0.05$ was considered significant.

RESULTS

Body Weight

Effect of extra virgin olive oil on the body weight of a female Wistar rats exposed to benzene and ethanol shows a significant increase ($p < 0.05$) in (EVOO) treated group when compared with the control group. However, there was a significant decrease in body weights of ETH, BEN, ETH + BEN, ETH+OLI, BEN+OLI and ETH+BEN+OLI groups when compared with the control.

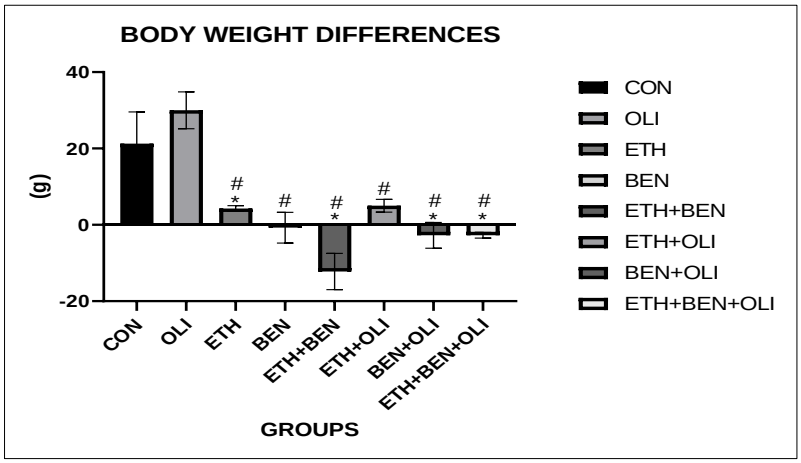

Fig. 1: A bar graph showing the body weight differences (g) across the groups.

*Values are Mean ± SEM of the data obtained; P values (P <0.05). *: indicates statistical significance when compared to control group at p< 0.05; #: indicates statistical significance when compared to olive oil at p<0.05.*

Organ Weight/Relative Organ Weight

Effect of extra virgin olive oil on organ and relative organ weight of a female Wistar rat exposed to benzene and ethanol shows a significant decrease ($p > 0.05$) in (EVOO) treated group when compared with the control group. This decrease was more pronounced organ weight of ETH, BEN, ETH + BEN, ETH+OLI, BEN+OLI and ETH+BEN+OLI groups when compared with the control but however shows a significant increase ($p > 0.05$) in the relative organ weight of ETH group when compared the normal control.

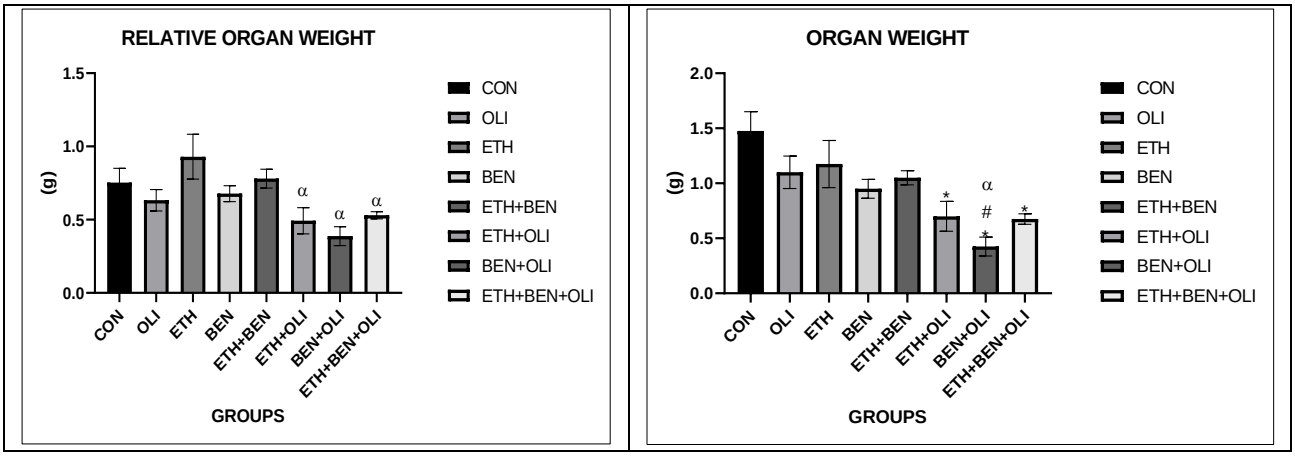

Fig. 2: A bar graph showing the Organ and Relative Organ weight (g) across the groups.

*Values are Mean ± SEM of the data obtained; P values (P <0.05). *: indicates statistical significance when compared to control; #: indicates statistical significance when compared to olive oil; α: indicates statistical significance when compared to ethanol.*

Progesterone

Effect of extra virgin olive oil on serum progesterone level of a female Wistar rat exposed to benzene and ethanol shows a significant decrease (p > 0.05) in (EVOO) treated group when compared with the control group. This decrease was more pronounced in ETH, BEN, ETH + BEN when compared with (EVOO) treated and the control group. There was a significant increase (p > 0.05) in ETH+OLI, BEN+OLI and ETH+BEN+OLI groups when compared with (EVOO) treated and the control group.

Extradiol

Effect of extra virgin olive oil on serum extradiol level of a female Wistar rat exposed to benzene and ethanol shows a significant increase (p > 0.05) in (EVOO) treated group when compared with the control group. This increase was more pronounced in ETH group when compared with (EVOO) treated and the control group. There was a significant increase (p > 0.05) in BEN, ETH + BEN, BEN+OLI and ETH+BEN+OLI groups when compared with when compared with the control group but ETH+OLI group shows a significant decrease in serum estradiol level when compared with (EVOO) treated group and the control.

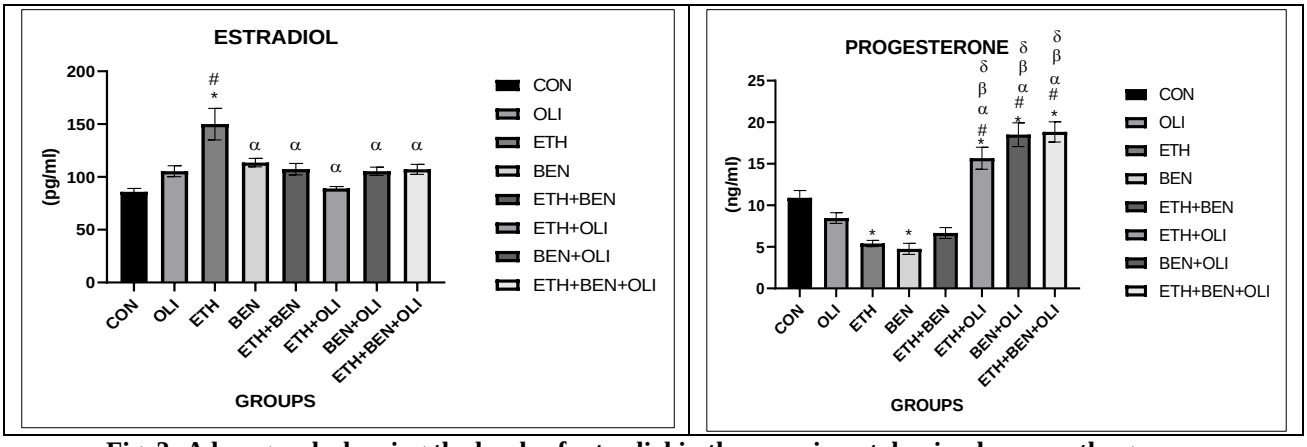

Fig. 3: A bar graph showing the levels of estradiol in the experimental animals across the groups

*Values are mean ± SEM of the data obtained; P values (P <0.05). *: indicates statistical significance when compared to control; #: indicates statistical significance when compared to olive oil; α: indicates statistical significance when compared to ethanol; β: indicates statistical significance when compared to benzene; δ: indicates statistical significance when compared to ethanol and benzene.*

HISTOLOGICAL EXAMINATION

The Control Group Plate 1 (Fig 4 and 5):

Shows a normal Photomicrograph of uterus treated with vehicle showing normal endometrium with endometrial glands and luminal epithelial cells (x 400).

EVOO Treated Group Plate 2 (Fig 4 and 5):

A normal Photomicrograph of uterus treated with extra virgin olive oil showing a normal endometrium, endometrial glands and luminal epithelial cells (x 400).

ETH + BEN Group Plate 3 (Fig 4 and 5):

A Photomicrograph of uterus treated with ethanol and benzene showing a hypertrophied endometrium, endometrial glands and luminal epithelium (x 400).

ETH + EVOO Group Plate 4 (Fig 4 and 5):

A Photomicrograph of uterus treated with ethanol and extra virgin olive oil showing slightly hypertrophied endometrium, endometrial glands and luminal epithelium (x 400).

ETH Group Plate 5 (Fig 4 and 5):

A photomicrograph of uterus treated with ethanol showing a hypertrophied endometrium, endometrial glands and luminal epithelium (x 400).

BEN Group Plate 6 (Fig 4 and 5):

A photomicrograph of uterus treated with benzene showing a hypertrophied endometrium, endometrial glands and luminal epithelium (x 400).

BEN + EVOO Group Plate 7 (Fig 4 and 5):

A Photomicrograph of uterus treated with ethanol and extra virgin olive oil showing slightly hypertrophied endometrium, endometrial glands and luminal epithelium (x 400).

ETH + BEN + EVOO group 8 (Fig 4 and 5):

A Photomicrograph of uterus treated with ethanol and extra virgin olive oil showing slightly hypertrophied endometrium, endometrial glands and luminal epithelium (x 400).

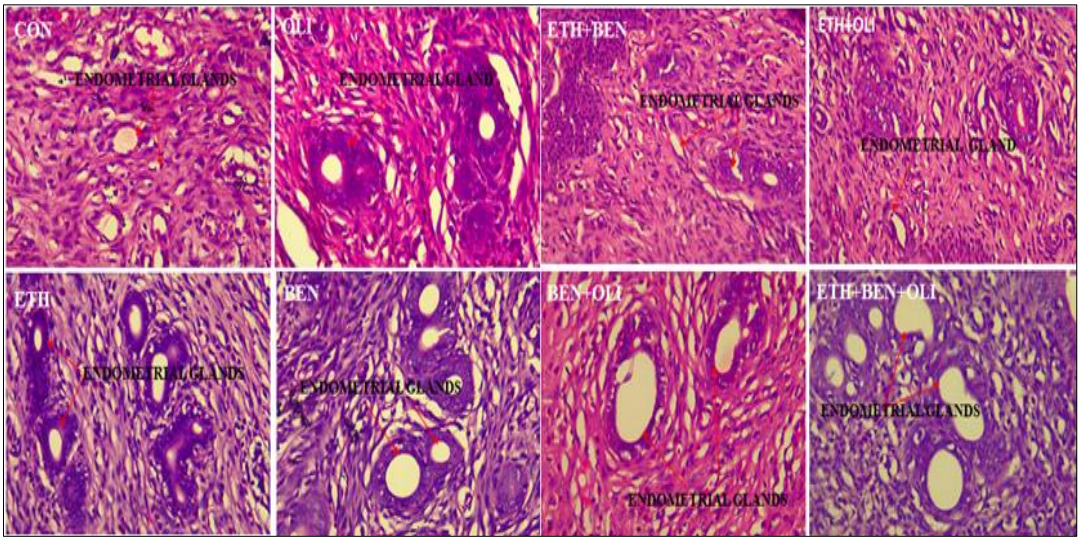

Fig. 4: Showing section of normal uterine tissue consisting of Myometrium, endometrial glands and artery (H&E X400)

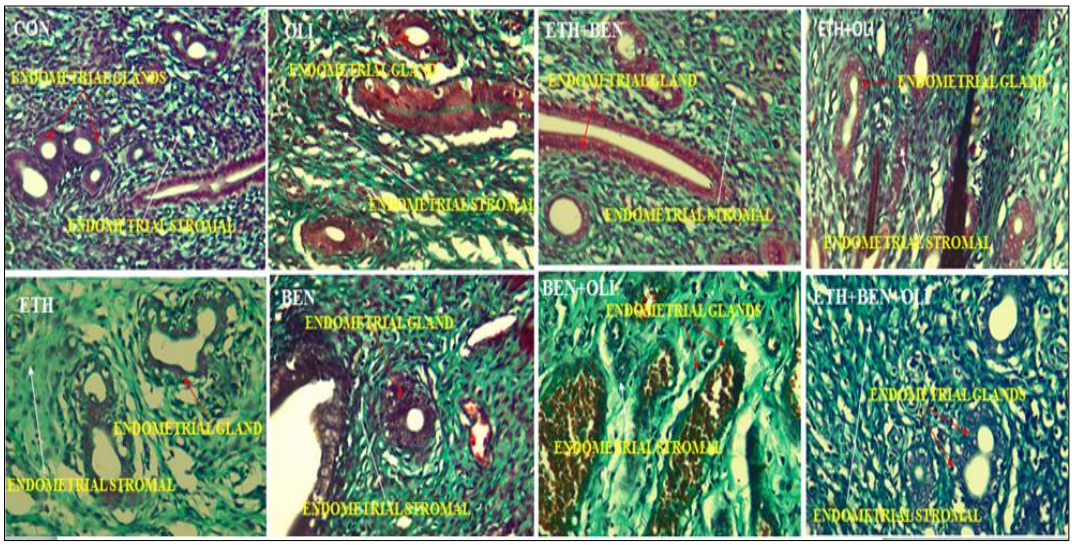

Fig. 5: Sections revealed endometrial stromal, endometrial glands and artery with remarkable fibrosis in the sections (MT X400)

DISCUSSION

The study provides important insights into the potential protective effects of EVOO against the detrimental impacts of benzene and ethanol exposure on the female reproductive system, particularly the uterus in an animal model.

The remarkable improvement in body weight of animals treated with extra virgin olive oil during treatment shown in (Fig 6) in this study. The results showed that EVOO treatment was able to significantly increase the body weight of the female rats compared to the control group. This effect was discussed in line with previous study of animals fed with high fat diet and extra virgin olive oil shows a similar increase in the high density lipid profile and in the case of Low density lipid profile, cholesterol and triglycerides which can be an evident in this study of an increase in body weight and metabolic parameters in the context of high-fat diet-induced obesity treated with EVOO [14]. The authors also suggested that the beneficial effects of EVOO on body weight may be attributed to its influence on lipid profiles and energy homeostasis. In contrast, the reduced body weights observed in the groups exposed to benzene and/or ethanol alone indicate the negative impacts of these chemicals on overall health and growth. Moreover, dietary habits with EVOO treatment are one of the most important contributing factors to the development and progression of body weight which can be an indicator of body weight improvement, The significant decrease in animal treated with benzene and ethanol in this study shows that according to previous study, the concentration of phenol in urine related to body weight (nmol/g b.w.) in control group and mice treated with ethanol, benzene and benzene + ethanol shows a significant decrease in body weight [15].

The study found that EVOO treatment maintained normal levels of the reproductive hormones progesterone and estradiol which were significantly reduced in the benzene and ethanol exposed groups. Additionally, benzene and ethanol altered steroid hormone levels in female rats where progesterone (Pg) and estradiol (E2) concentrations were significantly reduced as compared with control rats [4]. High benzene level in ovarian follicular fluid was associated with increased follicle stimulating hormone and decreased estradiol level in blood plasma [4]. This is an important finding as disruption of these key hormones can lead to various reproductive dysfunctions. It is evident that the antioxidant and anti-inflammatory properties of EVOO may be responsible for its protective effects on hormone homeostasis. Moreover, a study on effect of some plant oils on reproductive activities in female albino rats shows an increase in serum progesterone and serum estradiol level according to previous studies [16]. In this respect, a similar study by [17], reported that Oleic acid significantly increased the ratio of PGE2 to PGF2 which may contribute in granulosa cell differentiation and oocyte maturation in porcine and ewes, respectively. This was carried out to clarify sunflower oil supplementation maintain a normal ovarian function, oocyte recovery rate and maturation. This is supported by previous studies that have demonstrated the ability of EVOO components such as oleic acid and polyphenols to modulate steroidogenesis and ovarian function.

The histological examination revealed that benzene and ethanol exposure led to hypertrophy of the uterine endometrium, glands and luminal epithelium, indicating uterine dysfunction [4]. In contrast, the EVOO-treated groups showed a more normal uterine histology, suggesting that EVOO can help preserve the integrity and function of the uterine tissue. This protective effect of EVOO may be attributed to its anti-inflammatory properties as inflammation is a key contributor to uterine pathologies. The findings of this study are in agreement with previous literature on the adverse effects of benzene and ethanol on female reproductive health [3]. Benzene has been shown to disrupt ovarian function, reduce oocyte and embryo quality and increase the risk of ovarian tumors. Ethanol exposure has also been linked to alterations in the menstrual cycle, reduced fertility and impaired fetal development [3]. The current study extends these observations by demonstrating the detrimental impacts of these chemicals on the uterus.

CONCLUSION

This study provides evidence that EVOO with its rich composition and beneficial bioactive compounds can effectively mitigate the uterine dysfunctions induced by benzene and ethanol exposure. This suggests that EVOO may be a valuable therapeutic or preventive approach for managing reproductive health issues associated with exposure to these environmental pollutants. The mechanisms involving the antioxidant and anti-inflammatory properties of EVOO are well-supported by the existing literature on the health benefits of this Mediterranean dietary oil.

Acknowledgement: None

Conflict of Interest: None

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