ORIGINAL ARTICLE

ANTIOXIDANT VERSUS ENDOCRINE EFFECTS OF AVOCADO OIL IN BUTYLPARABEN-INDUCED OVARIAN DAMAGE OF PRE-PUBERTAL RATS

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ABSTRACT

Background: Butylparaben is used as preservative in cosmetics. Being structurally similar to estrogens, chronic exposure in consumers causes oxidative stress, ovarian damage and endocrine disruption. By adding avocado oil to diet, which has antioxidant as well as endocrine effects, this mitigation can be ameliorated to a great extent.

Objectives: To observe the ovarian histopathologic damage & endocrine disruption by butylparaben and whether such hazardous effects can be ameliorated by avocado oil, the antioxidant effects of which are to be compared with its endocrine effects.

Method: Thirty pre-pubertal female rats were divided into 6 groups of five rats each: A-Control, B-butylparaben exposed (dose: 10% of LD50-4.6 mg/kg b.w.), C-butylparaben exposed (dose: 40% of LD50-18.4 mg/kg b.w.), D-butylparaben exposed (10% of LD50-4.6 mg/kg b.w.) + avocado oil (dose: 4 mL/kg b.w.), E-butylparaben exposed (dose: 40% of LD50-18.4 mg/kg b.w.) + avocado oil (dose: 4 mL/kg b.w.) and F- avocado oil (dose: 4 mL/kg b.w.). Rats were sacrificed on day 31, ovarian tissue for histopathology & blood for hormonal assays taken.

Results: BP exposure enhanced estrogenic activity in group B & C due to which there were more atretic follicles in the ovaries and fewer primary, secondary, and corpus luteum follicles. Percentage weight gain was low, with high gonadal weight and GSI. FSH and LH levels were raised while E2 levels were low. On adding avocado oil, there was an increase in the number of secondary and tertiary follicle, weight gain increased and hormonal levels were comparable with control group.

Conclusion: Results confirm the potential ovarian histopathological damage and endocrine disruption by butylparaben, ameliorated by avocado oil, the antioxidant effects of which surpass its endocrine effects.

Keywords: butylparaben, preservative, gonadal damage, infertility, avocado oil benefits

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INTRODUCTION

Being colorless and odorless, parabens or butylparabens have a characteristic property of having chemical stability over a wide range of pH (4.5 to 7.5) and temperature, chemical inertness, low cost and water solubility. These characteristics play a key role in their demand as a commonly used preservative in pharmaceuticals and cosmetics (Lincho et al., 2021). Chemically, these alkyl esters of para-hydroxybenzoic

acid, have same structure as estrogen, so they interact with nuclear receptors, thus changing the process of steroidogenesis by altering the enzymes which metabolize these endogenous hormones, disrupting them thus affecting the reproduction. (Mitra et al. 2021; Boberg et al., 2020).

Due to common usage of cosmetics, shampoos, face masks, skin care products, lotions, nail-polish, perfumes, sun-blocks and hair gels, females are more exposed to paraben toxicity through the routes of dermal absorption, inhalation or ingestion. Women exposed to parabens, have 28–80% higher levels of total molar urine phthalate metabolites.

Parabens are considered not only as endocrine disrupting chemicals, thus weakening female fertility but also induce inflammatory and oxidative stress by inhibiting endogenous antioxidants (Ara et al. 2020). A study conducted in 2022 proved the risks associated with the exposure of phthalates or parabens in child-bearing-aged females through the use of personal care products via dermal absorption (Li et al., 2022). Paraben exposure leads to disturbed oocyte meiotic capacity and maturation due to mitochondrial dysfunction and accumulation of ROS with apoptosis thus decreasing the fertilization potential in females (Jeong et al., 2020; Guerra et al., 2023; Zhang et al., 2023).

To counteract parabens' hazardous effects, they should either be replaced by any other preservative or usage of any nutritive element like avocado oil should be added to diet. The usage of avocado oil as an upcoming healthy remedy to multiple disorders have been observed (Marques et al.,2022). Avocado oil being rich in MUFAs (monounsaturated fatty acids) and LSBC (lipid soluble bioactive compounds) like fatty acids, carotenoids, chlorophylls and tocopherols) play a key role as a strong antioxidant, cytoprotective and anti-inflammatory agent (Cervantes-Paz & Yahia 2021; Motta et al., 2021; Queiroz Junior et al., 2021). Avocado oil is well known for its high lipophilic nature with strong antioxidant and anti-inflammatory capacity. On the contrary, avocado fruit has also been reported as a traditional female contraceptive agent due to its extensive effects against hormones. Avocado extract has a stimulatory effect on anterior pituitary for the release of luteinizing hormone (LH) and follicle stimulating hormone (FSH) thus causing premature oocyte maturation (Obiandu et al., 2022). It also has a depressing effect on gonads thus negatively affecting the gonadal synthesis of estrogen/progesterone thus having antifertility effects (Cuschieri et al., 2023; Orabueze et al., 2021). In the present study, the antioxidant effects of avocado oil are to be compared with its endocrine antifertility effects in butylparaben-induced ovarian damage in pre-pubertal female rats and to observe whether the antioxidant effects of avocado oil ameliorate the inflammatory changes in ovary or the hormonal disturbance predominate thus causing the antifertility changes intact.

Hypothesis: Avocado oil protects against paraben-induced inflammatory ovarian damage and endocrine disruption.

Objectives:

1. To observe the paraben-induced ovarian histopathologic damage & endocrine disruption.
2. To study the ameliorative effects of avocado oil in such disruption.
3. To determine whether the antioxidant effect of avocado oil is predominant over its endocrine effect on butylparaben-induced ovarian damage in pre-pubertal rats.

Sample size & sample technique: n=30

Study design: Randomized experimental controlled trial

Place & duration of study: A study of four weeks held in the animal house-PGMI, Birdwood road, Lahore & SIMS, Lahore.

Inclusion & exclusion criteria:

Healthy, albino, female *Wistar* rats; Prepubertal age (25 & less than 25 days); Weight: 55-80 gm.

Exclusion-Female rats above 4 weeks as puberty hits at 30 days approx., Male rats

METHODS

A 30-days study in which 30 pre-pubertal female rats were divided into 6 groups of five rats each (random number generator). Out of these, group A was kept as control group while group B, C, D, E and F were experimental groups. All animals were given standard rat feed/ water *ad libitum*, 12-hr light-dark cycle maintained and doses given by oral gavage. Rats were sacrificed on day 31, 24-hours after last dose. Ovaries were removed and blood samples taken.

Butylparaben crystals to be used for solution preparation and Avocado oil (cold-pressed extraction) purchased from local market (Berhane et al., 2021 and Obiandu et al., 2022).

Table 1: Control & experimental groups.

Grp	Label	Butylparaben	Avocado oil
A	Control	Normal saline	--
B	Exp.	4.6 mg/kg b.w.	--
C	Exp.	18.4 mg/kg b.w.	--
D	Exp.	4.6 mg/kg b.w.	4 mL/kg b.w.
E	Exp.	18.4 mg/kg b.w.	4 mL/kg b.w.
F	Exp.	--	4 mL/kg b.w.

For histomorphological studies, ten sections per ovary were picked randomly and follicles were segregated into: Primary follicle (surrounded by a single granulosa cell layer), secondary follicles (few layers of granulosa cells but no antrum), antral follicles (with a clear antrum), atretic follicles (with degenerated oocyte & disorganized granulosa cells), empty follicles (multiple layers of granulosa cells but no oocyte) & Corpus luteum (formed by lutein cells & no ovum). Fig. 1 shows regular stages of primary, secondary, antral follicles and corpus luteum in control group A. While group B & C, shows a decreased number of primary, secondary follicles, corpus luteum; and increased number of atretic follicles. Groups D & E shows an increase in the number of secondary, tertiary follicles corpus luteum while Group F showed results comparable with control group A.

Statistical Analysis-Normality of data: The Shapiro-Wilk test was used to assess the normality of data regarding percentage change in body weight of animals from day 0 till day 30, weight of gonads, GSI, FSH, LH and E2 levels among groups. Table 3 presents a comparison of body weight and percentage change in body weight among six groups over 30 days, showing significant differences in initial body weight ($p=0.014$), body weight after 30 days ($p<0.001$), and percentage change in body weight ($p<0.001$). Group A, D and F show the highest percentage change in body weight (60.8 ± 3.8%, 54.1 ± 7.6% and 59.6 ± 11.0%, respectively), while Group C shows the lowest (22.1 ± 10.2%). Table 4 presents a comparison of weight of gonads and Gonadosomatic Index (GSI) among six groups, revealing that butylparaben significantly increased gonad weight and GSI ($p<0.001$). Group C shows the highest weight of gonads (0.07 ± 0.01) and GSI (0.106 ± 0.010), while Group A and F show the lowest GSI values (0.039 ± 0.029 and 0.040 ± 0.024, respectively). Table 2 shows serum female hormone levels, Group C had the highest FSH levels (7.88 ± 0.16) and LH levels (2.39 ± 0.60), and lowest E2 level (33.5 ± 2.6) while groups D & F showed levels comparable with the control group. Comparison of mean values of serum hormones among six groups (Table 5), revealed significant differences between them. Group C had the highest FSH levels (7.88 ± 0.16) and highest LH levels (2.39 ± 0.60), while Group A had the lowest FSH (1.38 ± 0.32). and LH (0.55 ± 0.10) level. Group A had the highest E2 levels (48.2 ± 1.3), while Group C had the lowest (33.5 ± 2.6). FSH concentration increased in group C significantly ($P \leq 0.001$). When avocado oil was added in groups D & E, FSH level became comparable with the control group. However, in the F group, treated with avocado oil only, hormone levels did not change.

DISCUSSION

The present study was conducted to particularize the reproductive toxicity and endocrine disruption due to butylparabens exposure in cosmetics and pharmaceutical products and to determine the ameliorative efficacy of avocado oil in such cases. In spite of their known and proved hazardous side effects, parabens are commonly used (Mitra et al. 2021).

Prepubertal exposure to low dose butylparaben delays the formation of primary follicles from primordial follicles leading to an increased number of atretic follicles and a decreased number of normal secondary follicles (Straczynska et al., 2022). As was previously observed in a related study, exposure to high doses of butylparaben increased the number of secondary follicles with a premature cavity (abnormal follicles) (Jeong et al., 2020). Accumulation of butylparaben in ovary not only directly inhibits the ovarian follicle development thus decreasing primordial follicles and increasing atretic follicles seen in a research work (Zhang et al., 2023) but also disturbs the hypothalamus-pituitary-gonadal-axis disrupting the hormones (Wang et al., 2024). Therefore, it is possible to speculate that Granulosa cells containing butylparaben may suppress folliculogenesis, lower the antral follicle survival rate, and produce more aberrant follicles. In addition to causing ovarian cycle disorders and delayed puberty onset, this also diminishes the ovarian reservoir of follicles, which in turn affects female fertility ((Li et al., 2022; Zhang et al., 2023).

Table 2: Hormonal assay through Eliza in female, *Wistar* prepubertal rats

Female hormone	A Control	B BP-4.6 mg/kg b.w.	C BP-18.4 mg/kg b.w.	D BP-4.6 mg/kg b.w. + AO- 4 mL/kg b.w.	E BP 18.4 mg/kg b.w. +AO- 4 mL/kg b.w.	F AO 4 mL/kg b.w.
FSH (mlU/ml)	1.38±0.03	4.67±0.45	7.88±0.33	1.89±0.98	1.69±0.04	1.58±0.05
LH (mlU/ml)	0.55±0.03	1.89±0.06	2.39±0.07	0.79±0.54	1.27±0.09	0.68±0.05
E2 (pg/ml)	48.74±0.36	39.03±0.66	33.65±0.41	41.32±0.38	42.98±0.53	43.79±0.49

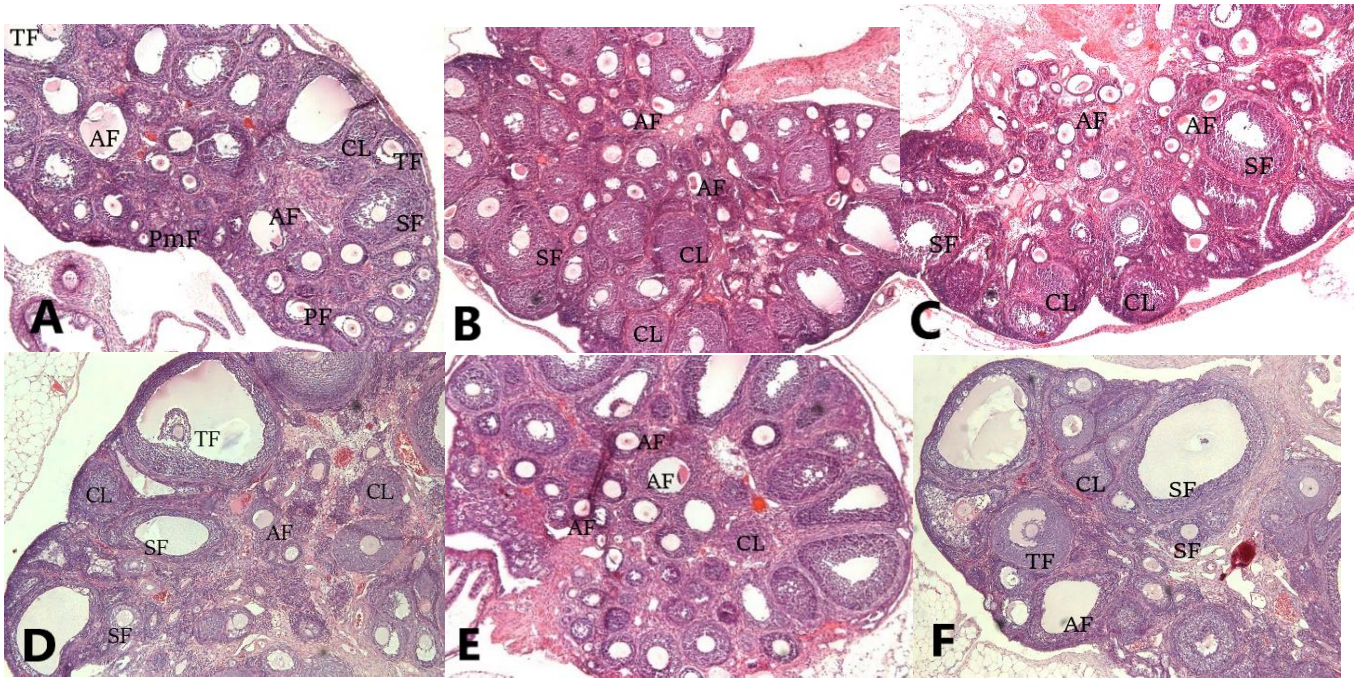

Fig.1 Histological sections stained with hematoxylin and eosin (magnification: 10X). Control group A exhibits the presence of follicles at different stages of maturity in the ovarian tissue. Follicles seen are primordial follicles (PmF), primary (PF), secondary (SF) & tertiary follicles with early antral development (TF) and Corpus Luteum (CL). There is clear discernment between the outer cortex with large tertiary follicles and medulla with numerous necrotic ova. The butylparaben-treated ovary (B & C) exhibits a deficient follicular reserve, preponderance of atretic follicles (AF) and many follicular cysts with absence of corpus luteum. Treatment with Avocado oil (D & E) reduces the number of follicular cysts.

Table 3: Comparison of body weight and percentage change among groups

Group	B. Weight at Day 0	B. Weight at Day 30	Percentage Change
Group A	55.0 ± 1.6	88.4 ± 1.1	60.8 ± 3.8
Group B	54.2 ± 2.3	72.5 ± 2.3	33.9 ± 8.1
Group C	56.4 ± 3.7	68.5 ± 1.9	22.1 ± 10.2
Group D	49.6 ± 2.5	76.4 ± 3.2	54.1 ± 7.6
Group E	53.6 ± 3.1	71.7 ± 3.3	34.3 ± 12.4
Group F	54.7 ± 2.6	87.2 ± 4.7	59.6 ± 11.0
p-value	0.014*	< 0.001*	< 0.001*

Table 4: Comparison of weight of gonads and GSI among groups

Group	Weight of Gonads	GSI
Group A	0.04 ± 0.03	0.039 ± 0.029
Group B	0.04 ± 0.01	0.053 ± 0.019
Group C	0.07 ± 0.01	0.106 ± 0.010
Group D	0.04 ± 0.03	0.047 ± 0.034
Group E	0.06 ± 0.01	0.088 ± 0.022
Group F	0.04 ± 0.02	0.040 ± 0.024
p-value	< 0.001*	< 0.001*

Table 5: Comparison of FSH, LH and E2 levels among groups

Group	FSH	LH	E2
Group A	1.38 ± 0.32	0.55 ± 0.10	48.2 ± 1.3
Group B	4.67 ± 0.41	1.89 ± 0.09	39.2 ± 0.8
Group C	7.88 ± 0.16	2.39 ± 0.60	33.5 ± 2.6
Group D	1.89 ± 0.09	0.79 ± 0.31	41.4 ± 1.1
Group E	1.69 ± 0.09	1.27 ± 0.22	43.0 ± 2.3
Group F	1.59 ± 0.18	0.68 ± 0.13	43.7 ± 2.4
p-value	< 0.001*	< 0.001*	< 0.001*

Data is expressed as means ± SD

However, as demonstrated by related studies, improvements were evident in groups that received butylparaben in addition to avocado oil (Cuschieri et al., 2023; Cervantes et al., 2021). According to research, avocado oil can effectively counteract the toxicities caused by parabens by altering the tissues' oxidative state (Marques et al., 2022). The most practical metric for identifying chemically induced toxicity in any experimental investigation is the animal's body weight. The present study demonstrated that butylparaben (BP) had a negative impact on rats' body weight gain, which was lower in the BP-exposed group than in the control group. The results were in accordance with Guerra et al. (Guerra et al 2022).

Organ toxicity is evident when the weight of the organ changes by more than 10%, especially when this change is accompanied by histomorphological alterations. The current study found that rats intoxicated with butylparaben had a marked increase in ovarian weight (Guerra et al., 2022). This was likely caused by inflammation brought on by butylparaben, excessive reactive oxygen species production, the proliferative ability of paraben esters, or the organs' ability to compensate for oxidative stress caused by butylparaben (Jeong et al., 2020).

FSH and LH levels significantly increased while E2 levels significantly decreased in the B and C groups, according to the hormonal assays. These results are consistent with those of Ara et al. (Ara et al., 2020).

Because of avocado oil's protective properties, the disrupted hormone levels were almost restored in the D + F groups; other studies also found a similar outcome (Obiandu et al., 2022). This proves that BP is an endocrine disruptor disturbing the hypothalamus-pituitary-gonadal axis. The present findings were similar to those of a similar study (Wang et al., 2024), proving that butylparaben causes a disruption of steroid hormones balance and consequent remedy by avocado oil (Motta et al., 2021; Queroz Junoir et al., 2021).

The analysis of results by using SPSS version 21.0. A *p*-value $\leq$ 0.05 was considered as statistically significant.

CONCLUSION

Natural oils taken as daily supplements are good for reproductive health. Butylparaben exposure in any form, during the early period of life not only disturbs the hormonal levels but also causes ovarian toxicity in females, while avocado oil plays a protective role against these noxious effects thus it can be regarded as a potential remedy in the reproductive toxicity.

LIMITATIONS

Selecting the similar age and weight for the study was a limitation. However, considering the highly significant difference between high and low dose butylparaben exhibiting different traits, it is possible to generalize this number of repetitions in only a small group of the community.

CONFLICT OF INTEREST

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

ETHICAL APPROVAL

Ethical approval was granted by Institutional Review Board, Services Institute of Medical Sieneses/ Services Hospital, Lahore. vide reference No IRB/2024/1343/Sims dated: 22/05/2024

CONFLICT OF INTEREST:

Authors declare no conflict of interest.

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AUTHOR'S CONTRIBUTIONS

LS: Concept design, analysis, critical analysis and manuscript writing
YB, HMA: Critical Review, manuscript writing
TF, SN: Data analysis and drafting
ALL AUTHORS: Approval of the final version of the manuscript to be published

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