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Estrogenic Effects of Phenylmercuric Acetate: A Uterotrophic Study in Ovariectomized Female Wistar Rats.

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ABSTRACT
Endocrine-disrupting chemicals (EDCs) interfere with hormonal signalling pathways and pose significant risks to reproductive health. Phenylmercuric acetate (PMA), an organomercury compound formerly used as a preservative and antimicrobial agent, has raised concerns due to its persistence, bioaccumulative nature, and potential endocrine-modulating properties. The present study evaluated the estrogenic activity of PMA using the uterotrophic bioassay in ovariectomized female Wistar rats. Animals were allocated into four groups (n = 6 per group): vehicle control (corn oil), positive control administered 17α-ethinyl estradiol (10 µg/kg, subcutaneous), and two PMA-treated groups receiving 2 mg/kg/day and 8 mg/kg/day orally for three consecutive days. Uterine wet weight, blotted weight, and uterine fluid content were assessed as primary endpoints, supported by histopathological examination of uterine tissue. PMA administration resulted in a dose-dependent and statistically significant increase in uterine weights and fluid content compared with controls, with the high-dose group exhibiting pronounced uterotrophic responses. Histological analyses revealed epithelial proliferation, stromal remodelling, inflammatory infiltration, and glandular degeneration, with severity increasing at higher PMA doses. No mortality or overt clinical toxicity was observed, although reduced food intake and mild body-weight changes were noted at the highest dose. These findings demonstrate that phenylmercuric acetate exerts estrogen-like effects in vivo and induces significant uterine structural alterations. The study highlights PMA as a potential endocrine disruptor with reproductive toxicity implications, underscoring the need for stringent regulatory evaluation and further investigation into its long-term health effects
Keywords: Phenylmercuric acetate; Endocrine-disrupting chemicals; Estrogenic activity; Uterotrophic bioassay; Ovariectomized rats; Organomercury compounds; Reproductive toxicity.

1. INTRODUCTION:

Endocrine-disrupting chemicals (EDCs) represent a broad and heterogeneous collection of both naturally occurring and synthetic chemical compounds which are well known for their capacity to intervene with endocrine system, which orchestrates hormonal regulation essential for physiological homeostasis. These chemicals disrupt normal endocrine function through multiple mechanisms, including antagonizing endogenous hormones, altering hormone synthesis and metabolism, modifying hormone receptor activity and affecting hormone transport and distributes pathways. Specifically, EDCs may bind to nuclear receptors such as receptors of estrogen (ERα and ERβ) androgen receptors (AR) and progesterone receptors (PR), either activating or blocking normal hormonal regulation related signaling pathway, reproduction, metabolism and behavior. Beyond classical receptor interactions, EDCs also exert effects via epigenetic modulation and interference with steroidogenic enzymes underscoring the multi-faceted and complex nature of their biological actions (Schug, 2016).

The escalating scientific and public health interest in EDCs stems from their prevalent presence in environment, consumer products and the food chains coupled with the revelation that many EDCs can exert effects at exceedingly low doses, often challenging traditional toxicological paradigms that rely on monotonic dose-response assumptions. Emerging evidence suggests that these low-dose and even nonmonotonic dose-response effects complicate risk assessments and demand innovative mechanism-based frameworks to better characterize and manage exposure-related risks (Diamanti Kandarakis, 2009).

Health implications related to EDC exposure are diverse and affect multiple organ systems across developmental stages. Reproductive disorders such as infertility, disrupted puberty timing and gonadal dysfunction are among the most studied adverse outcomes. However, EDCs also disrupt neuroendocrine functions leading to cognitive impairments and behavioral anomalies, contribute to metabolic disturbances like obesity, diabetes, thyroid dysfunction and promote the development of hormone-dependent malignancies

including breast, prostate and ovarian cancers. These multifaceted outcomes involve complex crosstalk between genomic and nongenomic signaling pathways, rendering the toxicological interpretation and translation into human risk challenging but essential for public health protection (Diamanti Kandarakis, 2009).

Among the diverse classes of EDCs, parabens, phthalates, bisphenols and heavy metals such as mercury have allured extensive attention towards the research due to their widespread use and demonstrated endocrine-modulating properties. Parabens, the alkyl esters of p-hydroxybenzoic acid which is widely used as preservatives, exhibit weak estrogenic effects modulated by their alkyl chain length and bioavailability. Phthalates, plasticizers common in various consumer products, disrupt reproductive development predominantly through interference with steroidogenic pathways. Bisphenol A (BPA), a high-production volume of chemical constituent of polycarbonate plastics and also the epoxy resins will acts as a potent xenoestrogen influencing multiple endocrine routes. Heavy metals, especially mercury and its organic derivatives such as phenylmercuric acetate (PMA), constitute a distinct group marked by persistence, bioaccumulation and complex toxicodynamic including receptor binding, enzyme inhibition and oxidative stress induction, thereby exerting unique endocrine-disrupting effects compared to organic EDCs (Eva, 2018).

Phenylmercuric acetate is an organomercury compound distinguished by a phenyl group bound to mercury via an acetate moiety, imparting considerable lipophilicity. This chemical attribute enhances its capacity to penetrate cellular membranes and bioaccumulate particularly in lipid-rich tissues such as endocrine glands. Historically, PMA was widely employed as a preservative and antimicrobial agent in pharmaceuticals and cosmetics due to its efficacy against contamination. However, increasing evidence of neurotoxicity, environmental persistence and bioaccumulation led to regulatory restrictions and phase-out especially in developed regions. Nevertheless, residual environmental contamination and ongoing use in less-regulated contexts preserve human exposure risks that are particularly concerning through unsafe periods like fetal development and early childhood due to PMAs ability to cross biological barriers and accumulate in target tissues (Wu, 2024).

Mercury can exist in elemental, man-made and organic chemical forms, each differing substantially in absorption, distribution, metabolism and toxicological profiles. Organic mercury compounds like PMA display higher lipid solubility, enabling wider tissue distribution including in critical endocrine target sites and the nervous system, which is what drives everything their elevated toxicity relative to other species. Their strong association with sulfhydryl groups allows binding to crucial proteins, steroidogenic enzymes, perturbing hormone biosynthesis and receptor signaling. Furthermore, PMAs oxidative stress induction contributes to cellular damage and epigenetic alterations that exacerbate endocrine disruption and may produce long-term multigenerational effects (Wu, 2024).

A comparative viewpoint reveals both PMA and parabens engage with estrogen receptors, disrupting hormonal homeostasis through molecular mimicry and receptor modulation. Nonetheless, PMA carries heightened systemic toxicity, encompassing neurotoxic, nephrotoxic and immunotoxin effects. This disruptive effect is largely attributable to mercury's chemical properties and oxidative stress mediation. This difference complicates public health risk assessments, invoking the need for compound-specific regulatory approaches that weigh not only endocrine-disrupting potential but also broader toxicological hazards. Consequently, regulatory frameworks incorporate PMA within persistent, bio accumulative and toxic substance (PBT) classifications, mandating stringent control and risk mitigation, whereas parabens are subject to risk assessments emphasizing endocrine endpoints and cumulative exposure (Clarkson, 2002).

In summary, the landscape of endocrine disruption encompasses structurally and mechanistically diverse agents, exemplified by PMA and parabens, whose differential chemical properties and toxicological profiles necessitate comprehensive and nuanced research and regulatory strategies. Understanding their specific molecular mechanisms, exposure routes and health implications is critical to informing predictive toxicology, risk assessment and public health policies aimed at mitigating adverse outcomes associated with endocrine-disrupting chemicals.

2. MATERIAL AND METHODS

Ethical Approval

The present research study protocol underwent stringent review and was approved by the Institutional Animal Ethics Committee (IAEC) of the Department of Postgraduate Studies and Research in Zoology at Sharnbasva University, Kalaburagi, Karnataka, India. This approval emphasizes the commitment to uphold the highest standards of ethical animal use consistent with both institutional policies and national legislation. The IAEC role centers on ensuring that all animal experimentation aligns with humane treatment and ethical justification, reflecting a balanced consideration of scientific goals and animal welfare. The institution is registered with the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India, providing formal regulatory oversight to animal experimentation conducted under its auspices. This registration, under the number 2236/PO/ReBiBt/S/23/CPCSEA, affirms compliance with national mandates aimed at regulating animal research practices. The ethical clearance, issued under reference number SUK/ZOL/IAEC/01/2024-25, signals adherence to standard operational procedures that govern research involving live vertebrates.

3. RESEARCH FRAMEWORK

The experimental design aimed to evaluate the estrogenic potential of phenylmercuric acetate (PMA) employing the uterotrophic bioassay, a well-established in vivo model for detecting estrogen receptor-mediated agonist activity. This bioassay is internationally recognized and widely

utilized for screening and hazard identification of endocrine-disrupting chemicals. The experiment was carried out in strict accordance with the Organization for Economic Co-operation and Development (OECD) Test Guidelines 440 published in 2001, a document that details standardized procedures to assess compounds for estrogenic activity in ovariectomized rodents. Complementing this, the study followed stipulations outlined by the Office of Chemical Safety and Pollution Prevention (OCSPP) guideline 890.1600 developed by the United States Environmental Protection Agency (EPA), thereby integrating dual international guidelines that guide best testing practices. The uterotrophic bioassay provides a robust framework for identifying substances that mimic estrogen by measuring uterine weight changes a sensitive endpoint reflective of estrogen receptor activation reinforcing the bioassay's mechanistic relevance and predictive validity (Owens, 2003). The use of such validated bioassays is crucial in regulatory toxicology for the both hazard screening and risk evaluation purposes, ensuring scientifically sound conclusions regarding the estrogenicity of chemical agents. Beyond methodological rigor, adherence to these international guidelines ensures harmonized data generation, facilitates data comparability and supports regulatory acceptance globally. Ethically, these protocols also embed harm-benefit analyses promoting minimal distress and use of animals by refining experimental approaches consistent with animal welfare regulations and societal expectations (Azilagbetor, 2024).

Test Compounds

The principal test compound employed was phenylmercuric acetate (PMA), a well-characterized organomercurial chemical with the CAS number 62-38-4 and a purity of 99.38% procured by Tokyo Chemical Industry (TCI), India Private Limited, Hyderabad. Its selection is grounded in prior evidence suggesting organomercurial compounds may exert estrogen-mimetic activities with toxicological implications. PMA was dissolved in proper solvent corn oil to facilitate identical dosing and administered orally at two predefined dose levels: 2 mg/kg/day as a low exposure group and 8 mg/kg/day representing a higher exposure setting. The dual-dose approach allows for the assessment of dose-response relationships which are critical for establishing toxicity thresholds and risk characterization.

The positive control, 17α-Ethinyl estradiol (EE; CAS No. 979-32-8; purity 99.28%), sourced from Sigma-Aldrich served as the gold-standard estrogenic agent for assay validation. EE was induced subcutaneously at the dose of 10 µg/kg, a dose well-established in literature to elicit robust uterotrophic responses. The vehicle chosen for both PMA and EE was corn oil, a standard inert medium supporting uniform compound dispersion. Administration of corn oil via oral gavage at a fixed quantity of 5 mL/kg of the body weight ensured consistent dosing volumes across groups, minimizing confounding variability related to dosing technique. The route of administration was deliberately selected based on pharmacokinetic considerations, with oral delivery modeling environmental exposures to PMA and subcutaneous injection of EE reflecting systemic delivery ensuring bioavailability (Farzaneh, 2016). The doses concord with previous toxicological investigations demonstrating estrogenic modulation by organomercurials (Cao, 2013).

Test Animals

Female Wistar rats were chosen owing to their widespread acceptance and validated use in reproductive and endocrine toxicology. These rats were purchased from National Institute of Nutrition (ICMR), Hyderabad, India ensuring a reputable source with documented animal health and genetic background consistency. Upon arrival, the rats were 8 weeks old, an age conducive to surgical interventions and experimental manipulation while providing standardization across the cohort. A thorough health examination was performed to confirm the absence of infectious or systemic disease that could potentially confound experimental outcomes. Animals were endowed a seven-day acclimatization period to the laboratory environment, a critical interval allowing physiological stabilization from transport stress and environmental adjustments influencing stress hormone levels, immune function and baseline reproductive hormone profiles (Chen, 2021). Post-acclimatization, the animals were put up in the animal house of the Department of Postgraduate Studies and Research in Zoology at Sharnbasva University, where environmental controls and husbandry practices furthered the maintenance of experimental integrity.

Animal Maintenance

The experimental animals were housed in polycarbonate cages, a material choice balancing durability and reduction of chemical leachates that might confound hormonal endpoints. Bedding comprised autoclaved rice husk was selected for minimal dust generation and low chemical interference, which cumulatively reduce respiratory irritants and environmental contaminants. These environmental parameters contribute to sustained animal welfare and data reliability. Room temperature was maintained at 23 ± 2°C and the relative humidity at 50 ± 10%, conditions empirically shown to stabilize physiological and endocrine responses in rodents. The 12-hour light and dark cycle was instituted to simulate natural circadian rhythms, crucial for hormonal regulation and behavioral patterns (Azilagbetor, 2024). Animals were grouped three per cage; a density optimized to balance social needs and minimize stress-induced confounders. Group assignments accounted for individual body weights to ensure that the average weights within groups did not vary by more than ±5 grams, an important step in reducing inter-group variability that could dilute treatment effects or inflate error margins (Cardoso, 2023). Identification for individual monitoring was achieved using tail markings applied with non-toxic markers, facilitating non-invasive recognition while minimizing distress associated with more intrusive marking techniques (Carvajal, 2018).

Feed and Water

Throughout the experimental period, animals received pelleted rodent chow from Champaka Feeds and Foods, Bangalore, India, administered *ad libitum*. The feed was composed following standard formulations supportive of the nutritional requirements of laboratory rodents, ensuring that any uterotrophic or systemic variations observed were not artifacts of dietary deficiencies or imbalances. Purified drinking water was available *ad libitum* via polycarbonate bottles to prevent mineral contamination or chemical interference associated with metallic or organotin water delivery systems common in other materials. Both feed quality and water purity are known to substantially influence reproductive hormone levels and metabolic activities; hence tight control over these variables contributes to the fidelity of bioassay results (McCloy, 2004).

4. OVARIECTOMY PROCEDURE

The experimental model necessitated ovariectomized rats as the uterotrophic assay endpoint critically depends on the elimination of endogenous estrogenic influence. Animals underwent bilateral ovariectomy at eight weeks of age under anesthesia, following the established procedural standard defined in OECD TG 440. The surgical approach involved a dorsolateral incision between the last rib and the iliac crest, chosen for minimized tissue trauma and optimal access to

ovaries. Each ovary was ligated carefully at its junction with the uterine horn and excised, thus effecting complete removal of endogenous estrogen production sources. The abdominal musculature and skin were sutured according to sterile surgical practices, with skin closure facilitated by auto-clips to expedite wound healing and reduce infection risk. A post-operative recovery period of 14 days was instituted, during which animals were monitored closely for complications such as infection, dehiscence or distress, ensuring sufficient hormonal washout and restoration of baseline physiology prior to the initiation of test substance administration (Owens, 2003). Animals were huddled together or in pairs during the recovery process to prevent social stress and promote wound healing. Analgesia was administered in accordance with institutional animal welfare guidelines (Milford, 2025).

Test Substance Administration

Following the animals were divided into different periods of recuperation. Four groups each comprising six rats per group to mitigate allocation bias, aligning sample sizes with OECD-recommended minimums to balance ethical use with statistical power (Cardoso, 2023). Group assignments included a control group receiveded only the corn oil orally, where as positive control category were administered 17α-Ethinyl estradiol (EE) subcutaneously at 10 µg/kg, a low-dose PMA group receiving 2 mg/kg/day via oral gavage and the high-dose PMA group receiving 8 mg/kg/day orally.

roup I	:	Control animals corn oil was fed with standard pellet and water
Group II	:	Positive control 10 µg/kg/day EE administered subcutaneously
Group III	:	Low-dose PMA 2 mg/kg/day by oral gavage, daily three days
Group IV	:	High-dose PMA 8 mg/kg/day by oral gavage, daily three days

Dosing was daily, reflecting continuous exposure conditions that mimic human environmental or occupational exposure scenarios. The oral route for PMA administration is particularly pertinent given common oral exposure pathways, while the subcutaneous route for EE ensures rapid systemic estrogenic activity necessary to validate the bioassay. The dosing quantity of 5ml/kg body weight has been standardized to minimize physiological variability related to administration volumes. Dose selection was informed by prior toxicological literature indicating PMAs potential to elicit estrogenic responses at these concentrations, ensuring both low and high exposure assessments (Cao, 2013).

Clinical Observations

Throughout the dosing period, animals were subjected to vigilant clinical monitoring. Daily observations were conducted focusing on general health parameters, checking for overt signs of toxicity such as changes in fur texture, skin lesions, abnormal postures and impaired locomotion. Twice-daily cage-side inspections during the working week allowed for early detection of pain-related behaviors or morbidity indicators, essential for timely intervention. Food and water intake were also monitored within observational assessments to detect anorexia or polydipsia, which could indicate

systemic distress or toxic effects. Behavioral assessments extended to noting any alterations in social interactions or stereotypies indicative of neurological or systemic dysfunction. Thorough documentation of these findings provided an ethical and scientific basis for assessing the tolerability of the interventions and the humane endpoints of the study (Milford, 2025). Such careful monitoring aligns with the ethical mandates of reducing distress and optimizing welfare in research animals (Carvajal, 2018).

Body Weight and Food Intake

Body weight of each animal was observed and noted daily using a precision electronic balance calibrated to 0.1 g accuracy, enabling detection of subtle fluctuations in weight that may signify toxicological or systemic effects of PMA or EE treatment. Concomitantly, feed consumption was quantified by meticulously measuring the difference between feed supplied and residuals, normalized per animal per day to accommodate varying group sizes within cages. This calculation provided an aggregate metric of food intake, correlate able to treatment-related effects on metabolism, appetite regulation and overall health. Tracking these parameters longitudinally from the first dosing day up to the day prior to necropsy permitted the construction of growth curves and feeding behavior profiles, critical for identifying adverse effects or nutritional imbalances induced by the

test compound or control treatments (Na, 2014). The integration of body weight data with food consumption patterns adds robustness to toxicity evaluations, as weight changes may reflect not only caloric intake alterations but also metabolic disruptions or malaise induced by xenobiotic exposure (Azilagbetor, 2024).

Uterine Weight Measurement

Twenty-four hours after final dose administration, all pf the experimental animals were humanely sacrificed by cervical dislocation, a method consistent with minimizing pain and distress while facilitating rapid death. Necropsy procedures were standardized, prioritizing rapid and careful dissection to remove the uterus and vagina. The uterus was excised and blotted gently on damp filter paper to remove intraluminal fluid, an essential step to avoid confounding weight measurements with variable fluid contents. The vagina was separately dissected at its junction with the uterine horn to permit independent weight assessments. Organ weights were recorded immediately using high-precision analytical balances with sensitivity to 0.1 mg, thereby ensuring precise quantification of estrogen-induced uterine growth as a primary endpoint. Tissue handling adhered to protocols preventing dehydration or mechanical damage before weighing, critical for reproducibility and accuracy in the sensitive uterotrophic bioassay (Owens, 2003). This precise tissue weighting forms the quantitative basis for subsequent statistical analysis of estrogenic responses.

Histomorphology Examination

Histological evaluation focused on a mid-portion segment of the uterus approximately 4 mm in length, sectioned perpendicular to the longitudinal axis to maintain anatomical consistency. Tissues were fixed using 10% neutral buffered formalin, a fixative well suited to preserving cellular architecture and facilitating downstream staining quality. Following fixation, standard histological tissue processing protocols were employed including graded ethanol dehydration, xylene clearing and embedding in paraffin wax blocks suitable for high-quality sectioning. Thin sections, 10 µm in thickness were cut with a rotary microtome (Weswox MT-1090 A) to facilitate optimal cellular resolution while maintaining structural integrity. Dewaxing took place with toluene, followed by rehydration with graded alcohols to water. Sections were stained with Ehrlich's hematoxylin, imparting nuclear contrast, counterstained with eosin for cytoplasmic visualization and subsequently mounted in DPX mounting medium for permanent preservation. Microscopic analyses were conducted using a Magnus microscope equipped with the Mag Cam Series-DC 5 imaging system, allowing for high-resolution visualization and digital photomicrography at 40x, 100x and 200x magnifications. These images provided detailed morphological assessment of uterine tissues, facilitating qualitative and quantitative evaluations of estrogenic histopathological changes such as epithelial height, stromal cellularity and vascular proliferation (Tappe-Theodor, 2022). This comprehensive histological characterization complements tissue weight metrics and enhances mechanistic understanding of compound effects.

Statistical Analysis

Quantitative data obtained from uterine and vaginal weights, body weight tracking and food intake data was analyzed statistically by using Graph-pad Prism software. Experimental datawas expressed as mean value accompanied by standard deviation (SD) providing a measure of central tendency and variability. Statistical comparisons among control and treated groups were performed through one-way analysis of variance (ANOVA), a robust method to ascertain group differences in parametric data sets. Following ANOVA, Dunnett's multiple comparisons test was applied to identify specific group deviations from the control cohort, critical in bioassays where the control provides the baseline for estrogenic activity assessment. The p-value having less than 0.05 was set as the criterion for statistical significance, ensuring rigor in differentiating true treatment effects from random variability. These analytical strategies are consistent with regulatory and scientific best practices in toxicology and pharmacology studies, supporting the validity and interpretability of the findings (Na, 2014).

5. RESULTS

Observation of General Clinical Signs

Throughout the entire duration of the research observation, no mortality was recorded in any of the groups investigated, indicating that the treatments administered did not induce acute lethality. Additionally, nope of the animals exhibited overt clinical signs of toxic effect or distress. Whereas the ovariectomized female rats across all treatment arms, including those administered phenylmercuric acetate (PMA) at both low and high doses as well as those receiving 17α-ethinyl estradiol (EE) maintained normal behaviors. These included consistent grooming patterns, stable postural tone and unimpaired locomotor activity suggesting maintenance of general health and absence of behavioral aberrations related to systemic toxicity. The lack of observable adverse clinical signs across treated groups implies that PMA and EE, under the study conditions did not exert immediate toxicological stress or cause any debilitating physical symptoms (Naciff, 2007), (Stoker, 2010), (Louis, 2017).

Changes in Body Weight

Initial measurements confirmed there were no notable dissimilarities in body weights across the experimental group, ensuring comparability at the study outset. Throughout the treatment period, group of animals administrated with high-dose PMA demonstrated a mild reduction in final body weight compared to baseline values. However, this depletion did not gain statistically significant, it just suggests subtle impact of the higher dose PMA on growth rates or metabolic processes, possibly reflecting early physiological adaptation or slight systemic stress. Similarly, the group administered EE exhibited a modest weight decrease, aligning with

expected estrogenic physiological modulation known to affect body mass regulation. Conversely, both control untreated group and low-dose PMA group which is maintained relatively stable weight profiles, reinforcing that lower exposure levels did not adversely affect growth parameters or general metabolism. These slight weight fluctuations, while not statistically conclusive, may serve as early indicators of systemic responses to the test substances, warranting further investigation to elucidate underlying metabolic pathways influenced by estrogenic compounds (Stoker, 2010), (Louis, 2017).

Food Consumption Patterns

Assessment of nutritional intake throughout the study revealed distinct patterns among the treatment groups. Rats exposed to high-dose PMA experienced a pronounced and statistically significant reduction in every day food intake commencing by the third day of dosing and continuing consistently until the studies conclusion. This sustained reduction in feeding behavior suggests systemic toxicity or stress induced by higher levels of PMA, potentially related to discomfort, metabolic disruptions or central appetite suppression mechanisms. Meanwhile, the EE-treated group exhibited a moderate but less marked decrease in food intake during the same time-frame, which aligns with estrogen's known anorectic effects reported in various animal models. Conversely the control and even low-dose PMA groups maintained steady food consumption without noticeable variability, indicating that these exposures did not provoke systemic disturbances sufficient to impair feeding behavior. These findings highlight food intake as a sensitive measure of systemic well-being and potential toxicological burden. Reductions in nutritional consumption can predispose or potentiate organ-specific toxicities by reducing caloric and nutrient availability, compounding physiological stress. The pronounced decline in the high-dose PMA group's food intake could therefore contribute to or exacerbate organ-targeted effects such as those observed in the uterus and warrants consideration in interpreting the broader toxicological profile of PMA (Stoker, 2010), (Louis, 2017).

Uterine Weight Changes

Administration of phenylmercuric acetate (PMA) for three consecutive days resulted in a dose-dependent increase in uterine wet weight, blotted weight and uterine fluid compared with the control group. In the control animals (corn oil), baseline uterine wet weight (71.2 ± 6.0 mg), blotted weight (61.2 ± 5.3 mg) and fluid content (10.0 ± 1.2 mg) were observed. The positive control group (EE, 10 µg/kg/day, s.c.) showed a marked uterotrophic response with significant increases in uterine wet weight (259.7 ± 20.7 mg), blotted weight (193.4 ± 11.4 mg) and uterine fluid (66.3 ± 11.0 mg) ($p < 0.001$ vs control). The low-dose PMA group (2 mg/kg/day, oral) demonstrated a modest but statistically significant increase in uterine wet weight (105.5 ± 8.7 mg), blotted weight (88.9 ± 7.5 mg) and uterine fluid (16.6 ± 2.1 mg) ($p < 0.05$ vs control). The high-dose PMA group (8 mg/kg/day, oral) exhibited a more pronounced effect with uterine wet weight (178.4 ± 15.3 mg), blotted weight (142.6 ± 12.7 mg) and uterine fluid (35.8 ± 4.6 mg) significantly higher than control ($p < 0.01$). These findings indicate that PMA exerts estrogen-like effects with responses proportional to the administered dose.

Histopathological Changes in Uterine Tissue

Microscopic examination of the uterine tissue revealed notable treatment-related alterations reflecting the degree of estrogenic stimulation and inflammatory response elicited by the agents tested. Control animals displayed normal uterine architecture characterized by intact mucosal epithelium, well-organized glandular structures and preserved stromal integrity, indicative of maintained physiological homeostasis. Administration of EE profoundly altered uterine histology, manifesting as classic estrogen-induced changes. These included pronounced epithelial hyperplasia, characterized by thickening and increased layering of the mucosal lining, interstitial edema signifying fluid accumulation, cystic degeneration within glandular structures and moderate atrophy of the submucosal glands. These features combine to indicate enhanced proliferative activity and tissue remodeling under estrogen influence.

In the low-dose PMA group, uterine alterations were more subtle but discernible. Mild vacuolar degeneration of mucosal epithelial cells was evident, indicating early cellular distress or disruption in structural integrity. Inflammatory infiltration by mononuclear and multinuclear cells was prominent, accompanied by increased connective tissue proliferation within the myometrium suggesting an incipient inflammatory response and stromal remodeling. These findings collectively reflect a low-level uterotrophic and inflammatory effect of PMA at submaximal doses.

At the highest PMA concentration, histopathological damage was markedly severe. There was multifocal loss of mucosal epithelial cells, extensive inflammatory cell infiltration throughout submucosal and myometrial layers and conspicuous degeneration of submucosal glands, indicating widespread tissue injury. These structural aberrations demonstrate that PMA induces a potent, dose-responsive uterine pathophysiology involving both proliferative and degenerative processes with concurrent inflammatory components. This pattern illustrates an aggressive uterotrophic and toxicologic reaction aligning with the compounds systemic effects observed in decreased food intake and subtle body weight reduction (Naciff, 2007), (Stoker, 2010). Together, these uterine histological observations elucidate the compounds dual role as both an estrogen-mimicking agent and a tissue irritant capable of eliciting inflammatory responses. The dose dependency seen reinforces the critical importance of exposure levels in modulating reproductive toxicity and potential risks associated with organomercurial compounds.

6. DISCUSSION

The findings from this uterotrophic assay robustly demonstrate that phenylmercuric acetate (PMA) exerts estrogenic effects in ovariectomized female rats in the

dose-dependent way. Inclusion of 17α-ethinyl estradiol (EE) as the positive control fall pivotal in establishing assay validity as EE triggered expected estrogenic responses such as significant raisein weight of uterus and the characteristic histological markers of estrogen stimulation, including epithelial proliferation and glandular remodeling. This outcome validates the sensitivity and appropriateness of the experimental design to discern estrogenic agents and serves as a benchmark against which PMAs effects can be compared (Stoker, 2010).

The experimental data reveal that exposure to higher doses of PMA mimics many uterotrophic effects characteristic of synthetic estrogens. The present study provides clear evidence that phenylmercuric acetate (PMA) possesses estrogenic activity, as shown by increased uterine weights and fluid accumulation in immature female rats. The uterotrophic assay is a well-established method for detecting estrogenic agonists and has been widely validated for endocrine disruptor screening (Ashby & Tinwell, 1998; Owens & Ashby, 2002). The positive control (EE) produced the expected strong uterotrophic response, confirming the sensitivity of the assay. In contrast, PMA produced weaker but significant effects consistent with its characterization as a weak estrogenic agent. The dose-dependent pattern observed here is in agreement with earlier findings on parabens and other weak endocrine-disrupting chemicals. For example, Kang *et. al,* (2000) reported that butylparaben induced a dose-related increase in uterine growth, while Byford *et. al,* (2002) demonstrated estrogenic activity of parabens in MCF7 cells. The observed increase in uterine wet and blotted weights suggests endometrial proliferation and stromal thickening, while elevated uterine fluid content indicates enhanced luminal secretion, both typical markers of estrogenic stimulation (Owens & Koëter, 2003). Although PMA did not induce effects as strong as EE, its significant activity raises concerns about its potential as an endocrine-disrupting compound.

Collectively, the results confirm that PMA induces estrogen-like uterotrophic responses in a dose-dependent method. These conclusions are not true important because they suggest that even weak estrogenic agents such as PMA could contribute to hormonal imbalance and reproductive toxicity upon chronic or high-level exposure.

7. CONCLUSION

In summary, this study furnishes compelling evidence that phenylmercuric acetate can induce dose-dependent estrogenic and inflammatory responses in the uterus of ovariectomized female rats. The high-dose PMA group exhibited significant uterine enlargement, epithelial proliferation and extensive histopathological disruptions akin to that induced by the synthetic estrogen EE. These effects spotlight PMAs role as a potent endocrine disruptor with significant reproductive toxicity ramifications. According to these results, there is a pressing need for comprehensive risk assessments and regulatory scrutiny concerning PMAs presence in the environment and occupational settings. Further research should prioritize elucidating its molecular mode of action, evaluating chronic exposure outcomes and exploring mitigative strategies to minimize PMA-associated health hazards (Naciff, 2007); (Stoker, 2010).

Table 4.1: The impact of 17-ethinyl estradiol and PMA at the doses of 2 and 8 mg/kg of the body weight variations in ovariectomized rats treated via subcutaneous injection.

Parameter	Control Corn Oil	EE 10 µg/kg	PMA 2 mg/kg	PMA 8 mg/kg
Initial Body Weight (g)	164 ± 14.68	170.33 ± 14.43	171.5 ± 16.56	157.5 ± 8.01
Final Body Weight (g)	167 ± 14.07	167.16 ± 14.68	168 ± 16.06	154.16 ± 8.15

Data are shown in format of Mean ±Standard deviation. Vehicle control has given with corn oil. Each treatment group used Six animals for the experimental study. All animals underwent ovariectomy at end of 8th week. After one week, the test substance was administered beneath the skin and orally once daily for three consecutive days. The animals were weighed and sacrificed by cervical dislocation 24 h after the last treatment. Significantly different from untreated controls at $p<0.05$. and * $p<0.01$.

Table 4.2: Effects of 17-ethinyl estradiol and PMA at doses of 2 and 8 mg/kg on the Food consumption variations in ovariectomized rats treated via subcutaneous injection.

Dosing Day	Control Corn Oil	EE 10 µg/kg	PMA 2 mg/kg	PMA 8 mg/kg
Food Consumption (g/day/ animal)				
1-2	4.2	4.8	4.6	4.20
2-3	4	3.6	3.5	3.29
3-final	4.1	2.2	2.1	1.72**

ANOVA complemented with one tailed Dunnett *p< 0.05 compared vehicle. E. Estradiol -10 µ/kg; PMA -2 mg/kg; IBP 8 mg/kg. *P<0.05, **P<0.01

Table 4.3: Effects of Phenylmercuric Acetate (PMA) on Uterine Wet Weight, Blotted Weight and Uterine Fluid Content in OVX Female Rats

Group	Treatment	Uterine Wet Weight (mg)	Uterine Blotted Weight (mg)	Uterine Fluid (mg)

I	Control Corn oil	71.2 ± 6.0	61.2 ± 5.3	10.0 ± 1.2
II	Positive Control EE 10 µg/kg	259.7 ± 20.7 ***	193.4 ± 11.4 ***	66.3 ± 11.0 ***
III	PMA 2 mg/kg/day	105.5 ± 8.7 *	88.9 ± 7.5 *	16.6 ± 2.1 *
IV	PMA 8 mg/kg	178.4 ± 15.3 **	142.6 ± 12.7 **	35.8 ± 4.6 **

Values are expressed as Mean ± SD. *** highly significant increase compared to control ($p < 0.001$), *mild but significant difference vs control ($p < 0.05$), ** moderate to strong significant increase vs control ($p < 0.01$).

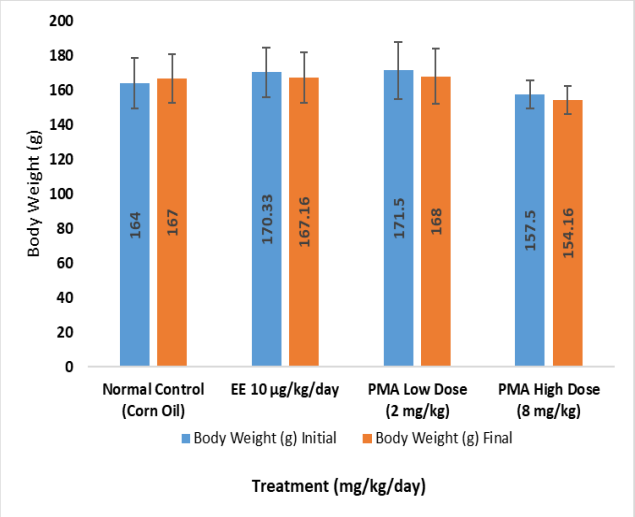
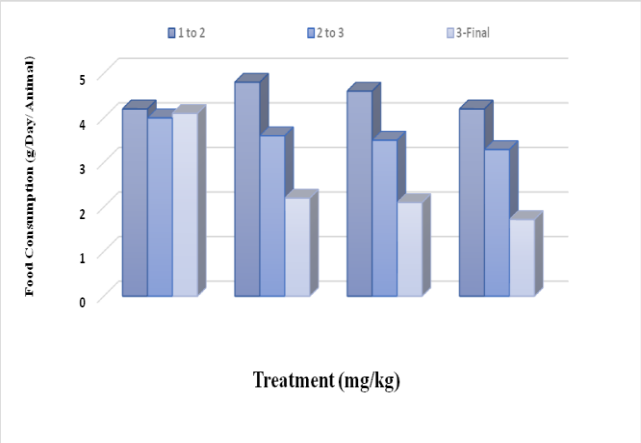


Figure 4.1: Impact of 17-ethinyl estradiol and PMA on body weight variations in ovariectomized rats



Control (Corn oil) EE 10 µ/kg/ PMA 2 mg/kg PMA 8 mg/kg

Figure 4.2: Effects of 17 Ethinyl estradiol and PMA at doses of 2 and 8 mg/kg on the food consumption

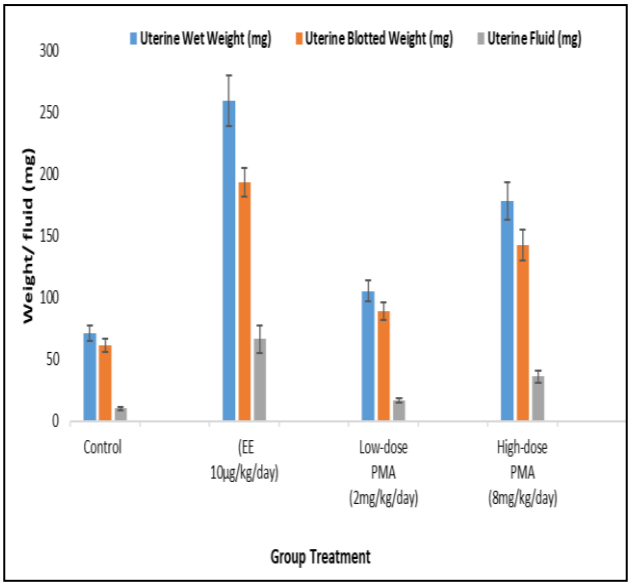
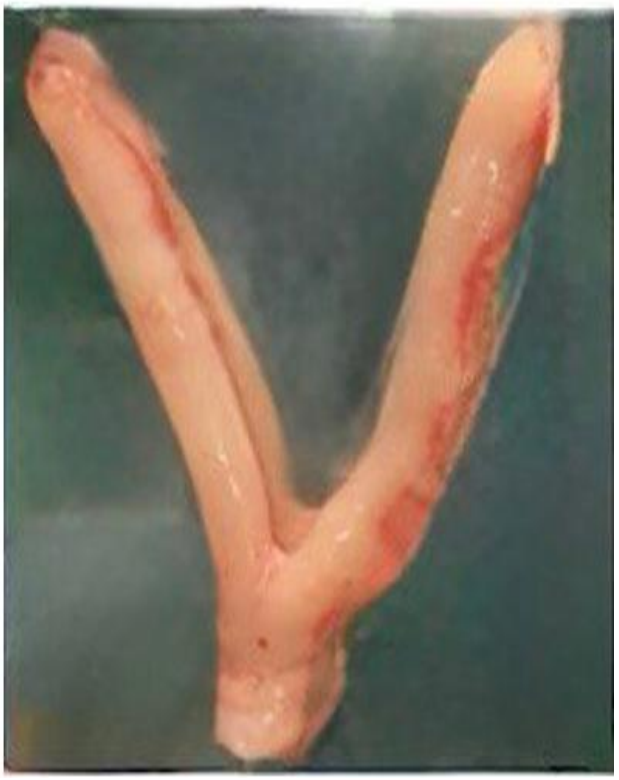
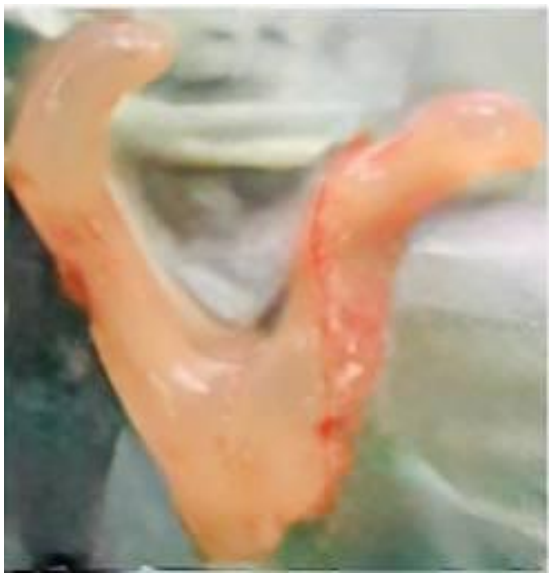
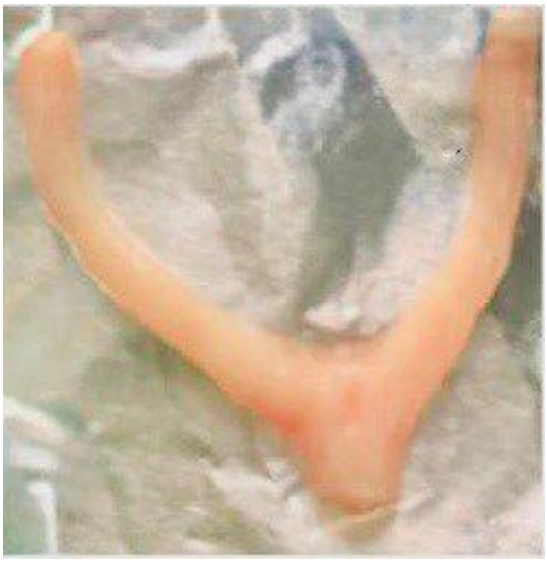


Figure 4.3: Dose-Dependent Uterotrophic Response to Phenylmercuric Acetate (PMA) Compared with Ethinyl Estradiol in Immature Female Rats







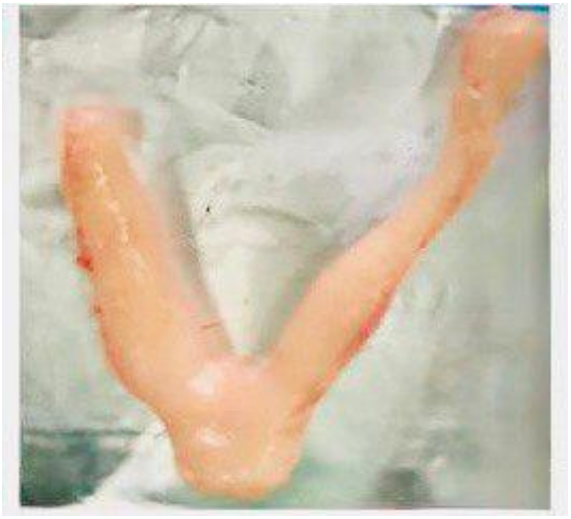


Figure 4.4: Photographs showing the uterine appearance in different treatment groups.

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