Oxidative Effects of 3,4-Methylenedioxymethamphetamine (MDMA) on the Ovaries and Uterus of Adult Female Wistar Rats: Implications for Female Reproductive Health

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Abstract

Background:
3,4-Methylenedioxymethamphetamine (MDMA), a common recreational drug, is known for neurotoxicity, but its effects on female reproductive organs are less understood. Oxidative stress may mediate its toxicity in non-neural tissues.

Materials and Methods:
Thirty adult female Wistar rats were divided into three groups: control, MDMA 80 mg/kg, and MDMA 160 mg/kg. MDMA was administered orally for 28 days daily. Post-treatment, ovarian and uterine tissues were analyzed for oxidative stress markers (SOD, CAT, GPx, MDA), and remaining rats were mated to assess fertility. Data were analyzed using one-way ANOVA and LSD post hoc tests ($p \leq 0.05$).

Results:
MDMA significantly disrupted oxidative balance in reproductive tissues. Group C showed the greatest reduction in antioxidant enzymes (SOD, CAT, GPx), while lipid peroxidation (MDA) peaked in Group B. Uterine tissues showed a similar oxidative pattern.

Conclusion:
MDMA induces dose-dependent oxidative stress in the ovaries and uterus, posing potential risks to female reproductive health and fertility.

Keywords:MDMA-induced oxidative stress; Female reproductive toxicity; Ovarian and uterine damage; Antioxidant enzyme activity; Wistar rat model.

Introduction

3,4-Methylenedioxymethamphetamine (MDMA), a synthetic entactogen widely used in recreational settings, is increasingly recognized for its systemic toxicological effects beyond the central nervous system.[1,2] While neurotoxicity remains the most studied consequence of MDMA exposure, emerging findings reveal its potential to disrupt peripheral organ systems, including the female reproductive tract. This isa concern given the drug's popularity among individuals of reproductive age.[3]

MDMA induces the generation of reactive oxygen species (ROS) through hepatic metabolism and mitochondrial disruption.[4]The resulting oxidative stress, defined by an imbalance between pro-oxidant load and antioxidant capacity, is a well-established mediator of tissue injury.[5] In the reproductive system, oxidative stress contributes to compromised oocyte quality, follicular atresia, and endometrial dysfunction, which in turn affect fertility, embryo implantation, and pregnancy outcomes.[6,7]

Ovarian tissue is particularly vulnerable due to high rates of cellular turnover and the oxidative sensitivity of granulosa cells and oocytes.[8] Simultaneously, the uterus, particularly the endometrium, relies on tight redox control to sustain receptivity and support early pregnancy.[9] Disruption of this balance by pharmacological agents has been linked to implantation failure, spontaneous abortion, and reduced uterine plasticity.[7]

Although oxidative damage induced by MDMA has been demonstrated in the brain, liver, heart and kidney,[10,11] little is known about its specific effects on the ovaries and uterus. To date, few experimental studies have assessed biochemical changes in female reproductive organs following MDMA exposure at pre-gestation. This gap is critical given the widespread use of MDMA and the subtle nature of reproductive toxicity, which may manifest long after exposure.[12]

Therefore, this study aims to experimentally evaluate the oxidative impact of MDMA on the ovaries and uterus using adult female Wistar rats as a model. By quantifying oxidative biomarkers: malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), we provide new insights into the mechanisms underlying MDMA-induced reproductive dysfunction. Our findings may inform clinical and public health strategies for mitigating drug-related fertility risks.

Materials:

- MDMA (3,4-Methylenedioxy-methamphetamine) was provided by the National Drug Law Enforcement Agency (NDLEA).
- Adult female Wistar rats (180–220 g), animal feed, weighing scales, dissecting tools, tissue preparation apparatus, and distilled water for hydration.

Methods:

Determination of Antioxidant Enzyme Activities and Lipid Peroxidation

The activities of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), as well as the concentration of malondialdehyde (MDA), were measured in tissue homogenates to assess oxidative stress levels.

- **SOD activity** was determined using the method of Beauchamp and Fridovich,[13] which is based on the inhibition of nitroblue tetrazolium (NBT) reduction by superoxide radicals generated via the xanthine-xanthine oxidase system. Absorbance was read at 560 nm.

CAT activity was measured according to the method of Aebi,[14] where the decomposition rate of hydrogen peroxide (H_2O_2) was monitored at 240 nm.

One unit of catalase activity was defined as the amount of enzyme that decomposes 1 µmol of H_2O_2 per minute.

- **GPx activity** was assessed using the method described by Paglia and Valentine,[15] in which the oxidation of reduced glutathione (GSH) is coupled with NADPH oxidation in the presence of glutathione reductase. The decrease in absorbance at 340 nm was recorded.
- **Lipid peroxidation**, indicated by **MDA levels**, was measured using the thiobarbituric acid reactive substances (TBARS) method as described by Ohkawa et al.[16] The absorbance of the MDA-TBA complex was measured at 532 nm, and MDA concentrations were expressed inµmol/mg protein.

Animal Care and Experimental Design
Animals and Ethical Statement:
A total of 30 female Wistar rats (180–220 g) were obtained from the Department of Anatomy, University of Benin Animal House. All animals were kept in same atmospheric condition all through the research period. The rats were acclimatized for two weeks before treatment and housed in wire-meshed cages under standard laboratory conditions (12 h light/dark cycle, 22 ± 2 °C, 50–60% humidity) with ad libitum access to food and water. This study is part of a PhD thesis conducted at the Department of Anatomy, University of Benin, Benin City. The research protocol with animal experimentation was approved by the Research and Ethics Committee of the College of Medical Sciences, University of Benin (REC Approved Number: CMS/REC/2024/620), and conducted in compliance with the National Institutes of Health's Guide for the Care and Use of Laboratory Animals.[17]

Experimental Groups/Administration of Drug:
The study was divided into three groups: A = Control, B = 80mg/kg, and C = 160mg/kg. Each group had 10 rats. group B and C were treated with MDMA orally via gavage at doses of 80mg/kg and 160mg/kg body weight for 28 days, while the control group received distilled water. The calculated LD_{50} for MDMA was 316.2 mg/kg, determined via a pilot study following Lorke's method.[18]At the end of the treatment, the rats from each group were sacrificed, and oxidative stress analysis was done for the ovaries and uterus.

Statistical Analysis:
Data were analyzed using One-Way ANOVA followed by LSD post-hoc test. The results were expressed as mean ± standard deviation. A p-value ≤ 0.05 was considered statistically significant. All statistical analyses were performed using IBM SPSS Version 29.0.

RESULTS

Pregestational Oxidative Outcome

Table 1.1: Oxidative Study of the Ovary

Parameters	Group A	Group B	Group C	P-Value
SOD (U/mg prot)	2.18±0.09	2.39±0.04	1.60±0.16#	0.00*
CAT (U/mg prot)	0.46±0.02	0.19±0.01#	0.18±0.02#	0.00*
GPx (U/mg prot)	2.16±0.11	2.60±0.07	1.95±0.22	0.02*
MDA (nmol/mg prot)	0.72±0.05	1.76±0.20#	0.38±0.03	0.00*

The results are presented in tables; Data are represented as Mean ± SEM; (+) means weight gain; (-) means weight loss.

NB: Group A= control, Group B= 80mg/kg body weight, Group C= 160mg/kg body weight; # = intergroup differences, * = significant differences at P ≤ 0.05. *U/mg prot* = Units of enzyme activity per milligram of protein. One unit (U) is typically defined as the amount of enzyme that catalyzes the conversion of 1 micromole (µmol) of substrate per minute underspecified conditions. *nmol/mg prot* = Nanomoles of a substance per milligram of protein. This unit reflects the concentration or quantity normalized to protein content.

In Table 1.1, the activities of antioxidant enzymes (SOD, CAT, and GPx) and the level of lipid peroxidation marker (MDA) were evaluated across the three experimental groups. Statistical analysis using one-way ANOVA revealed significant differences among the groups for all measured parameters ($P \leq 0.05$). Post hoc analysis with LSD further identified the specific group differences.

Superoxide dismutase (SOD) activity was significantly lower in Group C (1.60 ± 0.16 U/mg protein) compared to Groups A and B (P = 0.00), indicating reduced enzymatic defense against superoxide radicals. Although Group B showed a slight increase in SOD activity (2.39 ± 0.04 U/mg protein) relative to Group A (2.18 ± 0.09 U/mg protein), this difference was not statistically significant.

Catalase (CAT) activity was markedly reduced in Groups B and C (0.19 ± 0.01 and 0.18 ± 0.02 U/mg protein, respectively) when compared to Group A (0.46 ± 0.02 U/mg protein), with P = 0.00.

Glutathione peroxidase (GPx) activity was significantly increased in Group B (2.60 ± 0.07 U/mg protein) compared to Group C (1.95 ± 0.22 U/mg protein), while Group A (2.16 ± 0.11 U/mg protein) showed no significant difference from either (P = 0.02).

Malondialdehyde (MDA) levels, an indicator of lipid peroxidation, were significantly elevated in Group B (1.76 ± 0.20 nmol/mg protein) compared to both Group A (0.72 ± 0.05 nmol/mg protein) and Group C (0.38 ± 0.03 nmol/mg protein), with P = 0.00.

Table 1.2: Oxidative Study of the Uterus

Parameters	Group A	Group B	Group C	P-Value
SOD (U/mg prot)	3.28±0.16	1.52±0.03$^{\#}$	2.60±0.24$^{\#}$	0.00*
CAT (U/mg prot)	0.40±0.01	0.28±0.04$^{\#}$	0.19±0.02$^{\#}$	0.00*
GPx (U/mg prot)	3.64±0.18	1.75±0.05$^{\#}$	2.94±0.15$^{\#}$	0.00*
MDA (nmol/mg prot)	0.76±0.07	0.39±0.01$^{\#}$	0.59±0.05$^{\#}$	0.00*

The results are presented in tables and charts. Data are represented as Mean ± SEM; (+) means weight gain; (-) means weight loss.

NB: Group A= control, Group B= 80mg/kg body weight, Group C= 160mg/kg body weight; $^{\#}$ = intergroup differences, * = significant differences at $P \leq 0.05$.

Table 1.2 showed the activities of antioxidant enzymes: superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), as well as the level of malondialdehyde (MDA), a marker of lipid peroxidation, were evaluated in the uterine tissues of the experimental groups. Statistical analysis using one-way ANOVA showed significant differences among the groups for all measured parameters ($P \leq 0.05$)* and post hoc comparisons using the Least Significant Difference (LSD)$^{\#}$ test identified specific intergroup differences.

SOD activity was significantly reduced in Group B (1.52 ± 0.03 U/mg protein) and Group C (2.60 ± 0.24 U/mg protein) compared to Group A (3.28 ± 0.16 U/mg protein), indicating diminished capacity for detoxifying superoxide radicals in the treated groups, particularly in Group B (P = 0.00).

CAT activity also showed a significant decline in Group B (0.28 ± 0.04 U/mg protein) and Group C (0.19 ± 0.02 U/mg protein) relative to Group A (0.40 ± 0.01 U/mg protein), with the lowest activity observed in Group C (P = 0.00).

GPx activity was markedly lower in Group B (1.75 ± 0.05 U/mg protein) and Group C (2.94 ± 0.15 U/mg protein) compared to Group A (3.64 ± 0.18 U/mg protein), with the reduction more pronounced in Group B (P = 0.00).

Conversely, MDA levels were significantly decreased in Groups B (0.39 ± 0.01 nmol/mg protein) and C (0.59 ± 0.05 nmol/mg protein)

compared to Group A (0.76 ± 0.07 nmol/mg protein) (P = 0.00).

Discussion

The present study investigated the oxidative stress status in ovarian and uterine tissues following exposure to two different groups: B and C, as assessed by changes in key antioxidant enzymes: superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and the lipid peroxidation marker malondialdehyde (MDA). The results demonstrated dose-dependent and non-linear disruptions of oxidative homeostasis in both ovarian and uterine tissues, with distinctive patterns of enzymatic responses and lipid peroxidation across the treatment groups.

In ovarian tissue, SOD activity showed a differential response: significantly decreased in group C, indicating weakened primary antioxidant defense, while slightly increased (though not significantly) in group B, possibly reflecting an early compensatory response to rising reactive oxygen species (ROS). This pattern is consistent with previous findings suggesting that low to moderate oxidative stress may induce SOD expression as an adaptive mechanism. In contrast, excessive oxidative insult leads to enzyme degradation or inhibition.[19,20]

Catalase activity was markedly suppressed in both MDMA-treated groups, with no significant dose-dependent difference. This suggests that MDMA impairs hydrogen peroxide breakdown irrespective of dose, likely through oxidative modification of the enzyme or depletion of its essential cofactors. The disruption of CAT function, which operates downstream of SOD, implies a risk of hydrogen peroxide accumulation, thereby compounding oxidative stress. These findings are aligned with those of Zakaria et al. (2018),[21] who reported MDMA-induced catalase inhibition in neural and hepatic tissues.

Glutathione peroxidase exhibited a biphasic response. GPx activity was significantly elevated in group B, indicating an adaptive antioxidant response to neutralize accumulating peroxides. However, in group C, GPx activity declined markedly, likely due to glutathione depletion or enzyme inhibition under severe oxidative conditions. This finding is consistent with Brigelius-Flohé and Maiorino (2013),[22] who noted the vulnerability of GPx under glutathione-limiting stress, particularly in hormonally active tissues like the ovary.

MDA levels were significantly elevated in group B,suggesting increased lipid peroxidation resulting from inadequate antioxidant protection. Paradoxically, MDA levels were lowest in group C despite lower antioxidant enzyme activity. This unexpected result may indicate a collapse in metabolic activity or mitochondrial function at high MDMA doses, resulting in decreased Reactive Oxygen Species (ROS) generation or a loss of lipid substrates for peroxidation, similar to patterns reported by de la Torre et al. (2004).[23]

In uterine tissue, oxidative stress was also evident. SOD activity was significantly reduced in both MDMA-treated groups, suggesting compromised detoxification of superoxide radicals and increased susceptibility to oxidative damage. This mirrors observations by Cadet et al.,[24] who reported similar SOD impairment in neural tissues following MDMA exposure.

CAT activity was also significantly suppressed in the uterus across both treatment groups, further indicating compromised hydrogen peroxide detoxification. GPx activity was markedly reduced, particularly in group B, indicating impaired glutathione-dependent antioxidant defenses. This may be attributed to glutathione depletion or inhibition by MDMA metabolites, as described in earlier studies on amphetamine-induced oxidative toxicity.[25]

Interestingly, uterine MDA levels were significantly decreased in the MDMA groups, contradicting the usual association between oxidative stress and lipid peroxidation. Previous studies, such as those by García-Repetto et al.,[26] and Zakaria et al.,[21] reported increased MDA levels following MDMA exposure in other tissues. The discrepancy observed here may be due to a tissue-specific suppression of metabolic activity, early membrane damage reducing lipid peroxidation substrates, or apoptotic/necrotic processes diminishing oxidative reactions at the time of measurement. Taken together, these findings provide strong evidence that MDMA impairs redox balance in female reproductive tissues, with both compensatory and destructive responses depending on dosage and tissue type.

Conclusion

MDMA exposure exerts significant oxidative stress in both ovarian and uterine tissues, characterized by suppression of key antioxidant enzymes and complex patterns of lipid peroxidation. The ovarian response showed an initial adaptive phase at moderate doses, followed by enzymatic failure at higher doses. In the uterus, antioxidant systems are broadly suppressed, but the expected increase in lipid peroxidation was not observed, likely due to tissue-specific metabolic responses. These disruptions in oxidative homeostasis may contribute to impaired fertility, ovarian dysfunction, and broader reproductive toxicity.

Recommendations

- **Further Research**: Future studies should investigate mitochondrial function and apoptosis markers to better understand the full reproductive impact of MDMA.
- Future research should also evaluate the effects of MDMA on gene expression related to oxidative stress and apoptosis in reproductive tissues.
- **Public Health Awareness**: Given the popularity of MDMA as a recreational drug, public education campaigns should emphasize its potential long-term effects on female reproductive health.
- **Protective Strategies**: Investigation into antioxidant therapies or nutritional interventions may help mitigate the reproductive toxicity associated with MDMA.

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