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Effects of Amitriptyline on the Lipid Profile, Antioxidant, and Hormones of the first filial generation of Wistar Rats.

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ABSTRACT

Aim: This study investigated the effect of amitriptyline on the first filial generation of female Wistar rats by analyzing the anti-oxidant levels in serum and tissue, lipid profile, and reproductive hormones. **Methodology:** Twenty-five adults female Wistar rats (12 weeks) weighing between 150 and 180g were grouped into five (n=5). Group 1 (control) received 0.2 ml/kg of normal saline solution 28 days before and throughout the gestational period, group 2 and 3 received (5 and 10mg/kg) of amitriptyline respectively, 28 days before and throughout the gestation period; Group 4 and 5 (5 and 10mg/kg) of amitriptyline respectively for 28 days before the gestational period alone. After delivering, five female offspring rats from each group were allowed to grow naturally till puberty (12 weeks). Blood samples collected were analyzed for the level of GPx, Total cholesterol, Triglycerides, Low-density lipoprotein, High-density lipoprotein, estradiol, progesterone, prolactin, superoxide dismutase (SOD), Catalase (CAT), and Malondialdehyde (MDA) were assayed in the tissue homogenate and serum of the offspring. **Results:** Amitriptyline increases the MDA level of the offspring of the experimental animal, and decreases the level of SOD, CAT, and Estradiol. **Conclusion:** Amitriptyline induces oxidative stress, sexual dysfunction, and infertility in the F1 generation of Wistar rats.

تأثير الأميتريبتيلين على الدهون ومضادات الأكسدة والهرمونات في الجيل الأول من فئران ويستار

*أولواداري جيه أوغونديبي[1] و أبودونرين إيه أوجيتولا[1] و إيبوكون بي أوييبوا [2]و موبوا أو لاديل [1]و دينيس إس أروكويو[2] و أولاوالي أوبيمبي [2]و أومولولا إف أكينبيلو[3] و إستينشين أوسيريم[1] و داميلاري أفولابي[2] و أبيمبولا أو فاساكين[2] وأديولا إيه أياندوكون[2]

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الكلمات المفتاحية:
الأميتريبتيلين
مالوندهايد
كاتالاز
استراديول

الملخص

اجديت هذه الدراس بغرض تقييم تأثير الأميتريبتيلين على الجيل الأول من إناث فئران ويستار (Wistar) من خلال تحليل مستويات مضادات الأكسدة في المصل والأنسجة والدهون والهرمونات التناسلية. حيث تم في هذا البحث تقسيم خمسة وعشرين أنثى بالغة من فئران ويستار (12 أسبوعًا) يتراوح وزنها بين 150 و 180 جرامًا إلى خمس مجموعات (ن = 5). تلقت المجموعة 1 (المجموعة الضابطة) 0.2 مل / كجم من محلول ملحي طبيعي قبل 28 يومًا وطوال فترة الحمل، وتلقت المجموعة 2 و3 (5 و10 مجم / كجم) من الأميتريبتيلين على التوالي، قبل 28 يومًا وطوال

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فترة الحمل؛ المجموعة 4 و5 (5 و10 مجم / كجم) من الأميتريبتيلين على التوالي لمدة 28 يومًا قبل فترة الحمل وحدها. بعد الولادة، سُمح لخمس فئران صغيرة من كل مجموعة بالنمو بشكل طبيعي حتى سن البلوغ (12 أسبوعًا). تم تحليل عينات الدم التي تم جمعها لمعرفة مستوى GPx والكوليسترول الكلي والدهون الثلاثية والبروتين الدهني منخفض الكثافة والبروتين الدهني عالي الكثافة والإستراديول والبروجسترون والبرولاكتين وأكسيد الفائق ديسميوتاز (SOD) والكاتالاز (CAT)والمالونديالدهيد (MDA) في متجانسة الأنسجة ومصل النسل. النتائج: يزيد الأميتريبتيلين من مستوى MDA لنسل الحيوان التجريبي، ويقلل من مستوى SOD و CATوالإستراديول. الاستنتاج: يسبب الأميتريبتيلين الإجهاد التأكسدي والخلل الجنسي والعقم في الجيل الأول من فئران ويستار.

1. Introduction

Amitriptyline is a frequently used oral antidepressant with a serotonin re-uptake inhibition effect that increases the risk of certain organ-specific defects (1). It has a chemical formula of $C_{20}H_{25}N$ and a molecular weight of 277.403g/mol (2). It is a tricyclic antidepressant (TCA) class and its exact mechanism of action is unclear. Evidence suggest that it is more effective than another antidepressant including selective serotonin reuptake inhibitor (SSRIs) (3, 4). Depression is a life-threatening and psychiatric disorder, with the main symptom being feeling low incessantly. It is one of the most common complications of pregnancy. Associated with psychiatric disorders in pregnancy are; pre-delivery depression, recurrent depressive states, and postpartum depression. Antenatal depression has been associated with a high rate of poor pregnancy outcomes such as impaired fetoplacental function, decreased fetal growth, and neonatal complications (5, 6, 7, 8, 9). TCAs are expected to cross the placenta rapidly by passive diffusion because of their solubility in lipids. Neonatal withdrawal symptom has been reported in newborns following in-utero exposure to some TCAs. Amitriptyline crosses the placental at the rate of 8.2% (10).

This study is designed to investigate the effects of amitriptyline treatment on pregnant rats concerning the offspring of their first filial generation using the experimental rats' model.

2.0 Materials and Method

Chemicals used

The reagents that were used for the experiment include Bouins fluid, thiopentone, methylated spirit, Amitriptyline hydrochloride, Phosphate buffer solution, Normal saline solution, and Sodium thiopentone.

Equipment used

The equipment used for the experiment includes a dissecting Set, Digital weighing scale, Foil paper, Plain bottle, Cotton wool, Surgical gloves, Plain serum bottles, Dissecting Board, Bench centrifuge, Light Microscope, Slides and Cover slides, Centrifuge Machine, Microsurgical scissors, 1ml, 2ml, and 5ml Syringe, and Organ bottles.

Animals and Treatment

Twenty-five adult females (12 weeks) weighing between 150-180g were used in this study. The animals were procured and allowed to acclimatize for 2 weeks in the animal house of the College of Health Sciences at Osun State University, Nigeria. The animals were divided into 5 groups with each group consisting 5 rats. Group 1 received administered (0.2 ml/kg of normal saline solution) 28 days before and throughout the gestational period, Group 2; received (5mg/kg of amitriptyline) 28 days before and throughout the gestation period, Group 3 received (10mg/kg of amitriptyline) 28 days before and throughout the gestation period; Group 4 (5mg/kg of amitriptyline) 28 days before the gestation period alone; Group 5 (10mg/kg of amitriptyline) 28 days before the gestation period alone.

After delivering, five female offspring rats from each group were allowed to grow naturally till puberty (12 weeks).

They were exposed to normal rat chow and water *ad libitum*. In the experiment, adult male rats were introduced for mating. the animals were maintained on a 12-hour light-12-hour dark cycle under constant room temperature. Pairing for mating was 1:1 for males/females. vaginal smear was observed on the female rats to confirm that mating has taken place. The day after which either was found was considered as day 1 of gestation.

Serum and organ collection

Animals were sacrificed at 12 weeks under 0.3 ml/kg body weight of sodium thiopentone anesthesia. The animals were dissected (cut open from the abdominal cavity to the

thoracic cavity) Blood samples collected in plain serum bottles were centrifuged at 3000 revolutions per minute (rpm) for 15 minutes to obtain the serum. The serum is then used to carry out the lipid profile of the rats which are; Total cholesterol, Triglycerides, Low-density lipoprotein, High-density lipoprotein, the level estradiol, progesterone and prolactin using ELISA method with their respective hormonal assay kits, oxidative stress and the antioxidant enzymes (GPx, SOD, CAT, MDA) ovaries and uterus for each animal were collected, cleared of adherent tissues, weighed and recorded.

Determination of Lipid and lipoproteins

Total cholesterol, triglyceride, low-density lipoprotein cholesterol, and high-density lipoprotein

cholesterol levels were determined by enzymatic colorimetric method (11). The determination was based on the formation of color after enzymatic hydrolysis and oxidation.

The indicator quinoneimine used was formed from H202 and 4-amino-antipyrine in the presence of phenol. LDL cholesterol is estimated according to Friedewald's formula (12).

Estimation of Prolactin

The antibody-coated microplate was loaded with calibrators and samples. The prolactin in the sample binds to the antibodies fixed on the inner surface of the wells following incubation. The samples were washed to remove the non-reactive sample components. Afterward, a secondary polyclonal horseradish peroxidase-labeled antibody was added and incubated for 1-hour to form a sandwich complex consisting of the two antibodies and the rat prolactin. They are washed to remove excess enzyme conjugate. A chromogenic substrate, TMB (3,3',5,5'- Tetramethyl benzidine), is added to all wells and incubated for 30 minutes to form a colored end product (blue) by the fixed enzyme. The enzyme reaction is stopped by dispensing hydrochloric acid as a stop solution (change from blue to yellow). The optical density of the color solution is measured with a microplate reader at 450 nm.

Oxidative Stress Analysis

The tissues were homogenized in 10% phosphate buffer (100 mM) at a pH of 7.4 with the aid of an electric homogenizer (S1601001). The homogenates were centrifuged at 3000 rpm for 20 min and the

supernatants were collected for the assessment of the following indicators of oxidative stress and lipid peroxidation; Reduced glutathione (GSH) level was determined by the method of Beutler and co-workers (13), Super-oxide dismutase (SOD) by the method of McCord and Fridovich (14), catalase by the method of Sinha (15) while Malonaldehyde (MDA), an index of lipid peroxidation, was determined as described by Ohkawa et al. (16)

The atherogenic indices include Cardiac Risk Ratio (CRR), Atherogenic Coefficient (AC), and Atherogenic Index of Plasma (AIP). Cardiac Risk Ratio (CRR) was calculated by the ratio of total lipid cholesterol concentration to that of high-density lipoprotein. The atherogenic coefficient is the difference between total cholesterol and HDL cholesterol divided by HDL cholesterol while calculating the Atherogenic Index of Plasma (AIP) by using as previously described Eq. (17):

$$\text{Log} = \text{TG/HDL-C}$$

Statistical Analysis

Data collected were expressed as mean ± SEM. Statistical significance at $P \leq 0.05$ was determined using one-way ANOVA

3.0 Results.

Table 1: Effect of Amitriptyline on Tissue Antioxidant and Lipid Peroxidation in offspring of Wistar female rats

	Group 1	Group 2	Group 3	Group 4	Group 5
Serum GPx (iμ/ml)	2603 ± 53.98	2508 ± 20.44	1744 ± 44.11*$^{\alpha}$	2535 ± 2.48$^{\beta}$	1885 ± 58.31*$^{\alpha\beta\gamma}$
Serum SOD (iμ/ml)	2.26 ± 0.11	1.92 ± 0.09	1.88 ± 0.17	2.30 ± 0.06	1.90 ± 0.16
Serum Catalase (iμ/ml)	26.54 ± 1.18	20.96 ± 0.65*	19.20 ± 0.72*	28.10 ± 1.06$^{\alpha\beta}$	22.16 ± 0.65*$^{\gamma}$
Serum MDA (mmol/L)	25.92 ± 2.48	38.16 ± 1.14*	40.96 ± 1.44*	26.44 ± 3.18$^{\alpha\beta}$	37.62 ± 2.98*$^{\gamma}$

*= significant difference from control, α= significant difference from group 2, β= significant difference from group 3 and γ= significant difference from group 4

A significant decrease was recorded in serum GPx concentration in groups 3 and 5 (1744 ± 44.11 and 1885 ± 58.31) compared with control (2603 ± 53.98) and group 2 (2508 ± 20.44) ($p < 0.0001$, $F = 95.30$) also a significant difference in group 4 and 5 (2535 ± 2.48 and 1885 ± 58.31) compared to group 3 (1744 ± 44.11) while a decrease in group 5 (1885 ± 58.31) compared to group 4 (2535 ± 2.48).

The serum catalase concentration decreased significantly in groups 2, 3, and 5 (20.96 ± 0.65, 19.20 ± 0.72, and 22.16 ± 0.65) compared to the control (26.54 ± 1.18) ($p < 0.0001$, $F = 18.35$), also group 5 (22.16 ± 0.65) compared to group 4 (22.16 ± 0.65) while an increase in group 4 (22.16 ± 0.65) compared to group 2 (20.96 ± 0.65)

The serum MDA concentration increases significantly in groups 2, 3, and 5 (38.16 ± 1.14, 40.96 ± 1.44, and 37.62 ± 2.98) compared to the control (25.92 ± 2.48) ($p = 0.0003$, $F = 8.796$), also group 5 (37.62 ± 2.98) compared to group 4 (26.44 ± 3.18) while a decrease in group 4 (26.44 ± 3.18) compared to group 2 (38.16 ± 1.14)

Table 2: Effect of Amitriptyline on Tissue Antioxidant and Lipid Peroxidation in offspring of Wistar female rats

	Group 1	Group 2	Group 3	Group 4	Group 5
Tissue GPx (iμ/ml)	2628 ± 83.98	2390 ± 23.06*	1564 ± 33.13*$^{\alpha}$	2035 ± 2.48*$^{\alpha\beta}$	1825 ± 27.64*$^{\alpha\beta\gamma}$
Tissue SOD (iμ/ml)	1.94 ± 0.16	1.70 ± 0.08	1.60 ± 0.09	2.26 ± 0.15$^{\alpha\beta}$	1.80 ± 0.03$^{\gamma}$
Tissue Catalase (iμ/ml)	31.14 ± 1.21	23.64 ± 1.86*	21.08 ± 1.02*	28.76 ± 0.55$^{\alpha}$	23.90 ± 1.62*$^{\gamma}$
Tissue MDA (mmol/L)	28.10 ± 2.02	27.78 ± 1.73	41.86 ± 2.59*$^{\alpha}$	25.76 ± 1.05$^{\beta}$	32.04 ± 2.01$^{\beta}$

*= significant difference from control, α= significant difference from group 2, β= significant difference from group 3 and γ= significant difference from group

A significant decrease was recorded in tissue GPx concentration in group2, 3, 4, and 5 (2390 ± 23.06, 1564 ± 33.13, 2035 ± 2.48 and 1825 ± 27.64) compared with control (2628 ± 83.98) ($p < 0.0001$, $F = 96.43$) also in group 3, 4 and 5 (1564 ± 33.13, 2035 ± 2.48 and 1825 ± 27.64) compared to group 2 (2390 ± 23.06) and in group 5 (1825 ± 27.64) compare to group 4 (2035 ± 2.48), while an increase in group 4 and 5 (2035 ± 2.48 and 1825 ± 27.64) compared to group 3 (1564 ± 33.13).

The tissue SOD concentration increase significantly in group 4 (2.26 ± 0.15) compared to groups 2 and 3 (1.70 ± 0.08 and 1.60 ± 0.09) ($p = 0.0056$, $F = 5.046$) while a decrease in group 5 (1.80 ± 0.03) compared to group 4 (2.26 ± 0.15).

A significant decrease was recorded in tissue catalase concentration in groups 2, 3, and 5 (23.64 ± 1.86, 21.08 ± 1.02, and 23.90 ± 1.62) compared with control (31.14 ± 1.21) ($p = 0.0002$, $F = 9.529$) also in group 5 (23.90 ± 1.62) compared to group 4 (28.76 ± 0.55), while an increase in group 4 (28.76 ± 0.55) compared to group 2 (23.64 ± 1.86).

The tissue MDA concentration increased significantly in group 3 (41.86 ± 2.59) compared to groups 1 and 2 (28.10 ± 2.02 and 27.78 ± 1.73) ($p < 0.0001$, $F = 10.90$) while a decrease in groups 4 and 5 (25.76 ± 1.05 and 32.04 ± 2.01) compared to group 3 (41.86 ± 2.59).

Table 3: Effect of Amitriptyline on Tissue Lipid Profile in offspring of Wistar female rats

	Group 1	Group 2	Group 3	Group 4	Group 5
Total Cholesterol (mmol/L)	2.28 ± 0.04	2.68 ± 0.04*	1.96 ± 0.05*$^{\alpha}$	2.90 ± 0.07*$^{\alpha\beta}$	2.66 ± 0.09*$^{\beta\gamma}$
Triglyceride (mmol/L)	1.56 ± 0.05	1.62 ± 0.07	1.50 ± 0.10	1.82 ± 0.07$^{\beta}$	1.52 ± 0.06$^{\gamma}$
LDL (mmol/L)	1.06 ± 0.08	1.38 ± 0.06*	1.16 ± 0.16*	1.42 ± 0.15$^{\alpha}$	1.14 ± 0.13*$^{\gamma}$
HDL (mmol/L)	0.68 ± 0.04	0.72 ± 0.07	0.82 ± 0.05*$^{\alpha}$	0.70 ± 0.03$^{\beta}$	0.80 ± 0.03$^{\beta}$

*= significant difference from control, α= significant difference from group 2, β= significant difference from group 3 and γ= significant difference from group 4

A significant difference was recorded in total cholesterol concentration in groups, 3, 4, and 5 (2.68 ± 0.04, 1.96 ± 0.05, 2.90 ± 0.07, and 2.66 ± 0.09) compared with control (2.28 ± 0.04) ($p < 0.0001$, $F = 38.74$) also in group 3 and 4 (1.96 ± 0.05 and 2.90 ± 0.07) compared to group 2 (2.68 ± 0.04) and an increase in group 4 and 5 (2.90 ± 0.07 and 2.66 ± 0.09) compare to group 3 (1.96 ± 0.05), while a decrease in group 5 (2.66 ± 0.09) compared to group 4 (2.90 ± 0.07). The triglyceride concentration increased significantly in group 4 (1.82 ± 0.07) compared to group 3 (1.50 ± 0.10) ($p = 0.0432$, $F = 3.000$) while a decrease in group 5 (1.52 ± 0.06) compared to group 4 (1.82 ± 0.07).

Table 4: Effect of Amitriptyline on Hormonal Profile in offspring of Wistar female rats

	Group 1	Group 2	Group 3	Group 4	Group 5
Estradiol (pg/mL)	4.89 ± 0.27	4.88 ± 0.37	4.24 ± 0.24	3.83 ± 0.23*$^{\alpha}$	3.21 ± 0.11*$^{\alpha\beta}$
Progesterone (pg/mL)	1.57 ± 0.06	1.64 ± 0.03	1.54 ± 0.15	1.54 ± 0.08	1.37 ± 0.06
Prolactin (pg/mL)	0.87 ± 0.03	0.88 ± 0.15	0.81 ± 0.06	0.81 ± 0.03	0.69 ± 0.05

*= significant difference from control, α= significant difference from group 2, β= significant difference from group 3 and γ= significant difference from group 4

A significant decrease was recorded in estradiol concentration in groups 4 and 5 (3.83 ± 0.23 and 3.21 ± 0.11) compared with control and group 2 (4.89 ± 0.27 and 4.88 ± 0.37) (p = 0.0006, F = 7.708) also in group 5 (3.21 ± 0.11) compared to group 3 (4.24 ± 0.24).

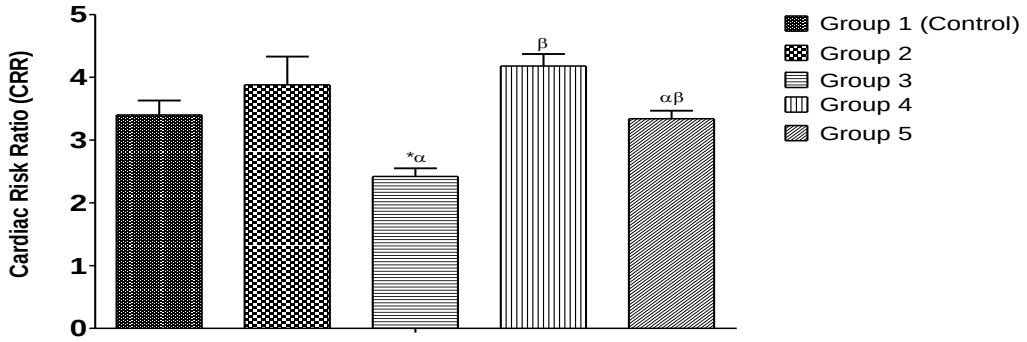

Fig 1:Effects of Amitryptiline on Cardiac Risk Ratio (CRR) in offspring of female Wistar rats

*= significant difference from control, α= significant difference from group 2, β= significant difference from group 3 and γ= significant difference from group 4

The Cardiac risk ratio decreased significantly in group 3 (2.42 ± 0.13) compared to groups 1 and 2 (3.40 ± 0.23 and 3.88 ± 0.45) (p= 0.0012, F = 6.893), while a significant increase in group 4 and 5 (4.18 ± 0.19 and 3.34 ± 0.13) compared to group 3 (2.42 ± 0.13).

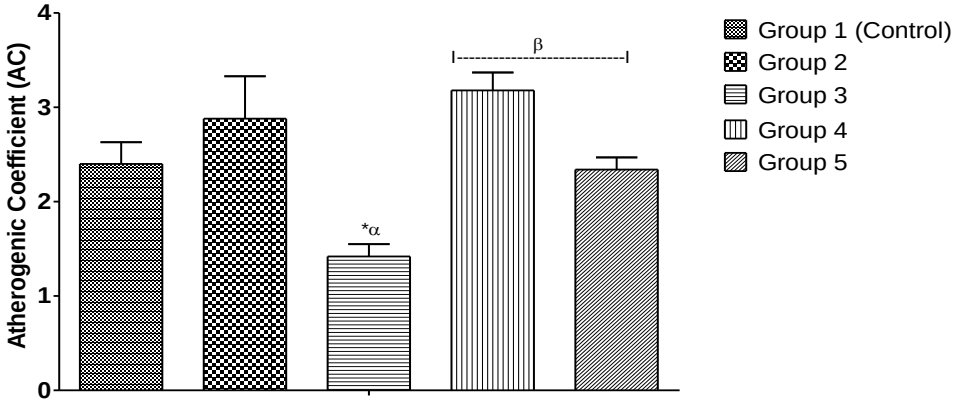

Fig 2:Effects of Amitryptiline on Atherogenic Coefficient (AC) in offspring of female Wistar rats

*= significant difference from control, α= significant difference from group 2, β= significant difference from group 3 and γ= significant difference from group 4

The Atherogenic coefficient decreased significantly in group 3 (1.42 ± 0.13) compared to groups 1 and 2 (2.40 ± 0.23 and 2.88 ± 0.45) (p= 0.0012, F = 6.893) while a significant increase in group 4 and 5 (3.18 ± 0.19 and 2.34 ± 0.13) compared to group 3 (1.42 ± 0.13).

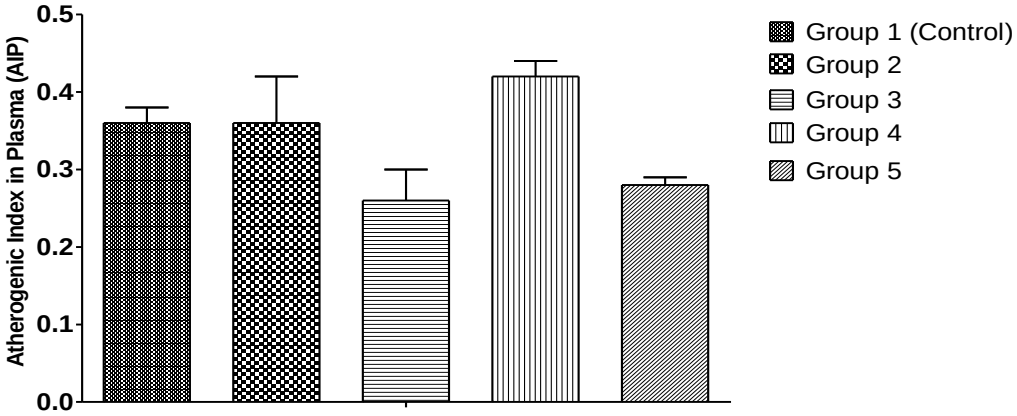

Fig 3:Effects of Amitryptiline on Atherogenic Index in plasma (AIP) in offspring of female Wistar rats

4.0 Discussion

This study investigated the effect of amitriptyline on the first filial generation of female Wistar rats by analyzing the anti-oxidant levels in serum and tissue, lipid profile, and reproductive hormones.

Malondialdehyde (MDA) is an important biomarker of oxidative stress. It is generated by the peroxidation of membrane polyunsaturated fatty acid (18). It is also produced in the process of prostaglandin synthesis (19). It is present as both monomer and higher-order oligomers, and its detection is considered to indicate lipid peroxidation. There was a significant increase in serum MDA of groups 2, 3, and 5 when compared with group 1. This increase in MDA level is an indication of oxidative stress in these groups. This

is a sign of imbalance between the production of Reactive Oxygen Species (ROS) and anti-oxidant defense which can cause damage to DNA, protein, and lipid, leading to Apoptosis or Necrosis in living cells (20).

Superoxide dismutases (SODs) form the front line of defense against reactive oxygen species (ROS)-mediated injury (21). These proteins catalyze the dismutation of superoxide anion free radical (O_2-) into molecular oxygen and hydrogen peroxide (H_2O_2) and decrease O_2- level which damages the cells at excessive concentration (22). From this study, the results show reduction though not significant in both the serum and the tissue SOD in groups 2, 3, and 5 when compared with group 1. This is an indication of oxidative stress in the experimental groups.

One of the most important antioxidant enzymes is the catalase. It is present in almost all aerobic organisms. Catalase breaks down two hydrogen peroxide molecules into one molecule of oxygen (23) and two molecules of water in a two-step reaction (24). Also, a significant decrease in catalase level was recorded in groups 2 and 3 when compared with group 1. This significant reduction could be a result of overutilization of the anti-oxidant to mop up free radicals produced during lipid peroxidation. It is a cellular anti-oxidant that helps to detoxify hydrogen peroxide to water and oxygen (25).

Glutathione peroxidase (Gpx) is an enzyme that reduces lipid hydroperoxides to the respective alcohol and hydrogen peroxide to water. The decrease in Gpx in the experimental group is an indication of hyperlipidemia

Hyperlipidemia is an increase level of lipids (fats) (Cholesterol, HDL, LDL, and triglycerides) in the bloodstream. They are transported in the blood as part of large molecules called lipoproteins (26). A high-cholesterol diet is an essential factor in developing cardiac diseases as it leads to hyperlipidemia, atherosclerosis, and ischemic heart diseases (27, 28). The potential for coronary heart disease (CHD) is increased in individuals with elevated concentrations of low-density plasma lipoprotein (LDL) Cholesterol (27). A significant difference was recorded in the lipid profile (Triglyceride, Cholesterol, HDL, and LDL) in all the experimental groups when compared with the control. Thus, indicating high lipid concentration in the offspring of experimental groups.

Atherogenic indices are used as an indicator of developing heart disease, the higher the index, the more the risk of developing heart disease (29, 30, and 31). Since there is no significant increase in these indicators, Amitriptyline poses no/ less risk of developing heart diseases in the offspring of the rats treated by it. This may be due to the duration of this study.

Amitriptyline doses have different effects on hormonal levels by disrupting the testosterone and estrogen ratio leading to sexual dysfunction and infertility (1)

The decrease in estradiol level observed in the experimental group when compared with the control group is evidence of epigenetic transfer as a result of fetal programming from the parent rats.

In conclusion, Amitriptyline induces oxidative stress, sexual dysfunction, and infertility in the F1 generation of Wistar rats

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