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Effects of *Peperomia pellucida* ethanol leaf extract on PAH-induced changes in lipid profile and reproductive parameters in male Wistar rats

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Abstract

The effects of ethanol leaf extract of *Peperomia pellucida* plant on Benzo[a]pyrene-induced changes in lipid profile and reproductive parameters in male Wistar rats was investigated. Thirty five (35) male rats weighing 150 –200g were divided into five (5) groups of seven (7) rats each. Ethanol extract of *P. pellucida* was prepared using air-dried leaves of the plant. Group-1 (negative control) received normal rat feeds and distilled water only for 6 weeks. Group-2 received 1 ml/kg b.w. of olive oil daily from week 1 to 3 (orally); Group-3 received 2mg/kg b.w. of B[a]P dissolved in 1 ml/kg b.w. olive oil (vehicle) daily from week 1 to 3 (orally); Group 4 (Curative group) received 2mg/kg B[a]P from weeks 1 to 3 before the administration of 500 mg/kg *P. pellucida* ethanol leaf extract from week 4 - 6. Group 5 (Prophylactic group) received 500mg/kg b.w of *P. pellucida* on alternate days from week 1 - 3 before administration of 2ml /kg b.w. B[a]P for another 3 weeks. Results obtained showed no significant ($p \leq 0.05$) changes in lipid profile in groups-1 and -2 at weeks 4 and 6. There was significant decrease in % fast progressive mobility of sperm cells in the Benzo-[a]P group which recorded 23.50%, in comparison with groups-1, -4 and -5 which recorded 87.00%, 67.5% and 62.00% respectively at week 4. Treatment with plant extract improved sperm count, viability, motility, and morphology. This result suggests that ethanol extract of *P. pellucida* leaves exhibit promising fertility effects and ameliorative potency against toxic effects of polycyclic aromatic hydrocarbon pollutants in Wistar rats.

Keywords: *Peperomia pellucida*; Benzo[a]pyrene; Lipid Profile; Reproductive Parameters; Leaf Extract

1. Introduction

Polycyclic Aromatic Hydrocarbons (PAHs) are a group of environmental pollutants that consist of two or more fused benzene rings regularly generated during incomplete combustion of organic matters [1]. PAH pollutants are found in aquatic and terrestrial ecosystems, as well as in the atmosphere [2]. Environmental PAHs can originate from natural sources, such as forest fires and volcanic emissions, and from sources associated with human activity (i.e., artificial or anthropogenic sources), such as coal burning, vehicles exhaust emissions, engine lubricating oils, and cigarette smoke [3]. Benzo[*a*]pyrene (B[a]P) is a Group 1 Agent whereas benz[*a*]anthracene (B[a]A), benzo[*b*]fluoranthene (B[b]F) and chrysene are classified as Group 2B Agents, all of which are carcinogenic to humans [4]. Gas-phase light PAHs present in the atmosphere can be adsorbed into particulate matter and, in conjunction with light and heavy PAHs accumulated in the soil and water, can enter the food chain through their uptake by vegetation and plant materials. Humans are exposed to PAHs through several routes such as air, water, food, skin contact, and occupational settings. For a large section of the general population not occupationally exposed to PAHs and atmospheric pollution, food ingestion is the major route of exposure compared to inhalation [5, 6].

Recent experimental studies have suggested that exposure to PAHs can significantly impair lipid metabolism and induce blood lipid elevation in mice [7-9]. However, epidemiological evidences on the impact of PAHs exposure on lipid metabolites are rarely reported. A previous study found that exposure to PAHs was positively associated with

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dyslipidemia in general adults through cross-sectional analysis [10]. Similarly, Wang *et al*. reported that exposure to polycyclic aromatic hydrocarbons (PAHs) is linked to abnormal lipid metabolism [11].

It also has been discovered that PAHs can influence reproductive efficiency in both males and females. There is evidence that exposure to PAH compounds has harmful effects on male reproductive health [12]. Overall, deleterious changes in the human reproductive system include gonadal insufficiency, cryptorchidism, hypospadias and testis cancer [13, 14]. It has been reported that the exposure to air pollution episodes of elevated PAHs was linked with a decline in semen quality, including asthenospermia, testicular germ cell carcinoma, abnormal morphology, urogenital tract abnormalities, and abnormal chromatin [15, 16]. Several studies also reported that exposure to PAHs or their metabolites has significant association with decreased sperm volume, sperm concentration, sperm abnormal morphology, sperm motility and with an increased risk of male idiopathic infertility [17 – 19].

One of the plants used as an alternative remedy for several diseases is *Peperomia pellucida*. This fleshy annual herb belongs to the *Piperaceae* family. Its common names are pepper elder, shiny bush, rat-ear, and man-to-man, and it is primarily found in tropical areas [20, 21]. In many Southeast Asian countries including Indonesia, this plant was believed to be efficacious for treating several diseases such as diabetes, muscle pain, aches, common cold and fever [22].

Available epidemiological data have shown links between environmental pollutants exposure and male reproductive health. Specifically, in recent years, isolated studies have been published regarding the potential link of PAHs exposure, changes in lipid profile and male infertility. As such, the aim of this study is to evaluate the relationship between PAHs exposure, lipid metabolism and male infertility.

2. Material and methods

2.1. Experimental Animals

Adult male Wistar rats weighing 150-200g were obtained from the Animal House, Department of Biochemistry, University of Port Harcourt Nigeria. The rats were allowed to acclimatize under standard conditions (25 ± 2 °C, 12 h of light and 12 h of darkness) for 2 weeks and then assigned randomly into five (5) groups of seven (7) rats each. The rats were housed in standard cages and given *ad libitum*, access to pelletized commercial rat feed and distilled water. Animal handling followed the procedures of the National Institute of Health Guide on the use of Animals for Experiments.

2.2. Chemicals

All reagents used in this study were of analytical grade: (Benzo[a]pyrene (B[a]P) with purity ≥ 96% high-performance liquid chromatography - CAS Number 50-32-8, B-1760, Chloroform, Distilled water, Olive Oil, Ethanol, Acetylene, and Assay Kits).

2.3. Preparation of Ethanol Leaf Extract of Peperomia Pellucida

Peperomia pellucida whole plant was obtained in Port Harcourt, Rivers State, Nigeria. The plant was identified and authenticated at the Department of Plant Science and Biotechnology, University of Port Harcourt, Nigeria. Fresh leaves of the plant were thoroughly washed with both tap water and distilled water. The leaves were air-dried in the shade at 33°C ± 2°C, ground into a fine powder using mechanical grinder and kept in air-tight jars. Aqueous extract of the plant leaves was prepared according to the method reported by Lemhadri *et al.* [23]. Fifty grams (50g) of dry powder was weighed into clean sterile bottles. The weighed dry powder was extracted using 250ml ethanol in tightly covered bottles and left for 48hours at room temperature. The resultant suspension was filtered into sterile beakers, and filtrates collected were refiltered using Whatman No.1 filter paper into sterile sample bottles. This were labelled appropriately and stored in plastic bags at -20 °C for the experiment.

2.4. Experimental Grouping

Experimental rats were administered Benzo[a]pyrene and *P. pellucida* ethanol leaf extract respectively. The PAH compound used in this study (B[a]p) was dissolved in olive oil which served as vehicle. Doses of *P. pellucida* (mg/kg) and B[a]P administered (mg/kg b.w) are as follows:

Table 1 Experimental Protocol

Experimental Group	Description
Group-I Normal Control	Received Normal Rat Feed + Water Only for 6 weeks.
Group II (Olive oil group)	Received 1 ml/kg b.w. of Olive Oil (vehicle) daily from week 1 to week 6 (orally).
Group –III (Negative Control)	Received 2mg/kg b.w. of B[a]P dissolved in 1 ml/kg b.w. Olive Oil (vehicle) daily from week 1 to week 6 (orally).
Group IV (Curative group)	Received 2mg/kg of B[a]P dissolved in 1 ml/kg b.w. Olive Oil (vehicle) daily from week 1 to week 3 (orally), and later treated with 500 mg/kg of *Peperomia pellucida* on alternate days (orally) from week 4 to 6.
Group V (Prophylactic group)	Received 500 mg/kg of *Peperomia pellucida* on alternate days from week 1 to week 3 after which they received 2mg/kg B[a]P (dissolved in olive oil) daily from week 4 to 6 (orally).

2.5. LD50 value for P. pellucida leaf extract and Benzo[a]pyrene/Olive Oil Dosage

LD_{50} value of 4000mg/kg b. w. was considered for *P. pellucida* leaf extract as previously reported by Desy *et al.* [24]. Doses administered for Benzo[a]pyrene were guided by a previous research [25] and the no-observed-adverse-effect level (NOAEL) & lowest observed adverse effect level (LOAEL) values as shown in Table 2, and sourced from the Danish Environmental Protection Agency [26]. Olive oil was administered following the dosage administered by [27].

Table 2. Summary of NOAELs/LOAELs from short-term toxicity tests of some PAH compounds following oral administration (gavage) [26]

Compound	Species	Duration	Critical Effect	NOAEL
Acenaphthene	mouse	90 days	Liver toxicity	175 mg/kg bw/day
Anthracene	mouse	90 days	None	1000 mg/kg bw/day (highest dosage)
Benzo[a]pyrene	Rat	90 days	Liver weight	3 mg/kg bw/day
Benzo[a]pyrene	rat	35days	Immunotoxicity	3 mg/kg bw/day
Fluoranthene	mouse	13 weeks	Liver/Kidney toxicity	125 mg/kg bw/day
Fluorene	mouse	13 weeks	Organ weight, haematology	125 mg/kg bw/day
Pyrene	mouse	13 weeks	Kidney toxicity	75 mg/kg bw/day

The experiment lasted for 6 weeks. The rats were fasted for 24 h at both weeks 4 and 6, prior to analytical sample collection. Blood samples were collected for biochemical analysis. The serum was separated by centrifugation at 3,000 g for 5 min and kept at −20°C until use for biochemical assays.

2.6. Biochemical Analysis / Assay for Hormone Levels (CRP, PSA, and testosterone)

2.6.1. Lipid Profile Analysis

Lipid Profile Serum total triglyceride concentration was measured by Tietz [28] method while serum total cholesterol level and serum HDL-cholesterol concentrations were analyzed using the methods described by Richmond [29] and Lopes- Virella *et al.* [30] respectively. Serum LDL-cholesterol level was calculated by the method of Friedewald *et al.* [31]. The PTS Pod combo test kit was used to assay for C-reactive protein (CRP), Prostate-specific antigen (PSA) and testosterone levels.

2.6.2. Semen collection and Analysis

Semen collection was done by the electro-ejaculation method [32]. Sperm motility, liveability, concentration and morphological abnormalities were evaluated using a modified method described by Ajani and Oyeyemi, Ajani *et al.* [33, 34].

3. Results and Discussion

Table 3 Effects of Ethanol Extract of P. pellucida leaf on Lipid Profile in B[a]P-Induced male Wistar rats

	CTR GRP-I (Control)	CTR GRP-II (Olive oil group)	CTR GRP-III (B [a]P group)	Group IV (Curative group)	Group V (Prophylactic group)
TC (mMol/L)					
Week 4	3.25 ± 1.45$^{abcde;fg}$	2.97 ± 0.33$^{abcde;fg}$	4.21 ± 1.11$^{abcde;fg}$	3.28 ± 0.88$^{abcde;fg}$	3.41 ± 1.10$^{abcde;fg}$
Week 6	2.96 ± 1.37$^{abcde;fg}$	3.00 ± 1.03$^{abcde;fg}$	4.21 ± 0.95$^{abcde;fg}$	3.18 ± 1.10$^{abcde;fg}$	2.24 ± 0.98$^{abcde;fg}$
TG (mMol/L)					
Week 4	1.23 ± 0.53$^{abcde;fg}$	1.00 ± 0.21$^{abcde;fg}$	1.27 ± 0.13$^{abcde;fg}$	1.37 ± 0.23$^{abcde;fg}$	1.15 ± 0.21$^{abcde;fg}$
Week 6	1.07 ± 0.48$^{abcde;fg}$	1.00 ± 0.12$^{abcde;fg}$	1.20 ± 0.11$^{abcde;fg}$	1.21 ± 0.41$^{abcde;fg}$	1.00 ± 0.11$^{abcde;fg}$
HDL (mMol/L)					
Week 4	1.34 ± 0.59$^{abcde;fg}$	2.00 ± 0.53$^{abcde;fg}$	2.51 ± 0.43$^{abcde;fg}$	2.36 ± 1.10$^{abcde;fg}$	2.22 ± 0.67$^{abcde;fg}$
Week 6	1.35 ± 0.59$^{abe;fg}$	2.02 ± 0.67$^{abcde;fg}$	2.51 ± 0.22$^{bcde;fg}$	2.38 ± 0.53$^{bcde;fg}$	1.98 ± 0.44$^{abcde;fg}$
LDL (mMol/L)					
Week 4	1.55 ± 0.67$^{abcde;fg}$	1.21 ± 0.11$^{abcde;fg}$	1.74 ± 0.12$^{abcde;fg}$	1.68 ± 0.23$^{abcde;fg}$	1.19 ± 0.71$^{abcde;f}$
Week 6	1.33 ± 0.58$^{abcde;fg}$	1.20 ± 0.41$^{abcde;fg}$	1.68 ± 0.10$^{abcde;fg}$	1.55 ± 0.32$^{abcde;fg}$	2.10 ± 0.79$^{abcde;g}$

Values are presented as mean ± SD of triplicate determination (N=3). Mean values with same superscript letters are not statistically significant at P ≤ 0.05 across the groups. Superscripts a, b, c, d, e show difference among groups while f, g shows difference among days. TC - Total Cholesterol, TG – Triglycerides, HDL – High-Density Lipoprotein, LDL – Low-Density Lipoprotein

Table 4 Effects of Ethanol Extract of *P. pellucida* leaf on Hormone Levels in B[a]P-Induced Wistar Rats

	CTR GRP-I (Control)	CTR GRP-II (Olive oil group)	CTR GRP-III (B [a]P group)	Group IV (Curative group)	Group V (Prophylactic group)
Testosterone (ng/ml)					
Week 4	1.91 ± 0.77$^{abcde;fg}$	1.70 ± 0.88$^{abcde;fg}$	2.31 ± 0.67$^{abcde;f}$	2.45 ± 0.40$^{abcde;fg}$	2.05 ± 0.57$^{abcde;fg}$
Week 6	1.90 ± 0.04$^{abce;fg}$	1.60 ± 0.31$^{abce;fg}$	1.33 ± 0.04^{abc,g}	2.68 ± 0.21$^{de;fg}$	2.28 ± 0.84$^{abde;fg}$

PSA (ng/ml)					
Week 4	0.11 ± 0.02$^{acde;f}$	0.10 ± 0.02$^{b;f}$	0.35 ± 0.02$^{acd;f}$	0.13 ± 0.04$^{acde;fg}$	0.14 ± 0.05$^{ade;f}$
Week 6	0.20 ± 0.01$^{ab;g}$	0.18 ± 0.04$^{ab;g}$	0.10 ± 0.01$^{cd;g}$	0.11 ± 0.02$^{de;fg}$	0.77 ± 0.03$^{e;g}$
CRP (ng/ml)					
Week 4	2.60 ± 0.51$^{abde;fg}$	4.61 ± 0.74$^{abde;fg}$	1.91 ± 0.05$^{c;f}$	2.75 ± 0.57$^{abde;fg}$	2.84 ± 0.21$^{abde;fg}$
Week 6	2.44 ± 0.72$^{ade;fg}$	5.00 ± 0.23$^{b;fg}$	0.53 ± 0.06$^{c;g}$	2.78 ± 0.31$^{ade;fg}$	3.00 ± 0.51$^{ade;fg}$

Values are presented as mean ± SD of triplicate determination (N=3). Mean values with same superscript letters are not statistically significant at P ≤ 0.05 across the groups. Superscripts a, b, c, d, e show difference among groups while f, g shows difference among days. PSA - Prostate-Specific Antigen, CRP – C-Reactive Protein

Table 5a Effects of Ethanol Extract of *P. pellucida* leaf on Sperm Quality Parameters in B[a]P-Induced Wistar Rats

	CTR GRP-I (Control)	**CTR GRP-II (Olive oil group)**	**CTR GRP-III (B[a]P group)**	**Group IV (Curative group)**	**Group V (Prophylactic group)**
Semen volume (%ml)					
Week 4	0.15 ± 0.03$^{abcde;fg}$	0.15 ± 0.04$^{abcde;fg}$	0.10 ± 0.02ab$^{cde;fg}$	0.20 ± 0.03$^{abcde;fg}$	0.20 ± 0.05$^{abcde;fg}$
Week 6	0.15 ± 0.05$^{abcde;fg}$	0.15 ± 0.12$^{abcde;fg}$	0.15 ± 0.03$^{abcde;fg}$	0.25 ± 0.04$^{abcde;fg}$	0.26 ± 0.03$^{abcde;fg}$
Sperm viability (%)					
Week 4	88.00 ± 3.65$^{a;fg}$	80.11 ± 5.21$^{b;fg}$	35.50 ± 1.22$^{c;fg}$	62.00 ± 5.55$^{d;fg}$	72.00 ± 4.41$^{e;fg}$
Week 6	88.00 ± 4.62$^{abe;fg}$	81.00 ± 6.23$^{ab;fg}$	30.00 ± 3.21$^{c;fg}$	62.00 ± 4.87$^{d;fg}$	75.50 ± 4.45$^{be;fg}$
Normal Sperm Morphology (%)					
Week 4	94.50 ± 8.41$^{ab;fg}$	91.00 ± 5.33$^{ab;fg}$	12.00 ± 1.25$^{c;fg}$	64.00 ± 3.69$^{de;fg}$	67.50 ± 5.65$^{de;fg}$
Week 6	93.50 ± 10.42$^{ab;fg}$	91.00 ± 3.89$^{ab;fg}$	11.00 ± 1.21$^{c;fg}$	65.00 ± 6.32$^{de;fg}$	67.50 ± 3.76$^{de;fg}$
Abnormal Sperm Morphology (%)					
Week 4	5.50 ± 1.21$^{ab;fg}$	9.00 ± 2.21$^{ab;fg}$	88.00 ± 4.45$^{c;fg}$	36.00 ± 4.32$^{de;fg}$	32.50 ± 3.88$^{de;fg}$
Week 6	4.50 ± 1.42$^{ab;fg}$	7.50 ± 2.23$^{ab;fg}$	86.78 ± 8.31$^{c;fg}$	36.00 ± 4.12$^{de;fg}$	33.30 ± 4.23$^{de;fg}$

Values are presented as mean ± SD of triplicate determination (N=3). Mean values with same superscript letters are not statistically significant at P ≤ 0.05 across the groups. Superscripts a, b, c, d, e show difference among groups while f, g shows difference among days.

Table 5b Effects of Ethanol Extract of *P. pellucida* leaf on Sperm Quality Parameters in B[a]P-Induced Wistar Rats

	CTR GRP-I (Control)	CTR GRP-II (Olive oil group)	CTR GRP-III (Benzo[a]pyrene group)	Group IV (Curative group)	Group V (Prophylactic group)
Fast progressive mobility (%)					
Week 4	87.00 ± 5.31$^{ab;fg}$	88.70 ± 6.31$^{ab;fg}$	23.50 ± 3.45$^{c;fg}$	67.50 ± 3.65$^{de;fg}$	62.00 ± 6.54$^{de;f}$
Week 6	86.00 ± 6.43$^{abe;fg}$	80.00 ± 4.22$^{abe;fg}$	23.50 ± 5.21$^{c;fg}$	66.00 ± 4.79$^{d;fg}$	78.21 ± 4.26$^{abe;g}$
Slow progressive mobility (%)					
Week 4	6.50 ± 1.25$^{ab;fg}$	6.00 ± 1.34$^{ab;fg}$	48.00 ± 1.44$^{c;fg}$	12.00 ± 2.21$^{de;fg}$	12.00 ± 1.12$^{de;fg}$
Week 6	4.00 ± 3.21$^{abd;fg}$	7.00 ± 3.21$^{abde;fg}$	50.50 ± 6.31$^{c;fg}$	9.00 ± 2.34$^{abde;fg}$	12.00 ± 3.12$^{bde;fg}$
Sperm Dead (%)					
Week 4	11.50 ± 1.25$^{ab;fg}$	11.80 ± 1.11$^{ab;fg}$	35.00 ± 5.52$^{cd;f}$	37.50 ± 4.21$^{cd;fg}$	47.60 ± 4.23$^{e;fg}$
Week 6	10.00 ± 1.32$^{ab;fg}$	11.30 ± 2.21$^{ab;fg}$	22.31 ± 3.33$^{c;g}$	36.50 ± 3.23$^{d;fg}$	47.60 ± 2.34$^{e;fg}$
Sperm count (10^5mL)					
Week 4	550.00 ± 45.45$^{ab;fg}$	545.00 ± 45.32$^{ab;fg}$	76.00 ± 7.21$^{cde;fg}$	85.00 ± 4.21$^{cde;fg}$	75.00 ± 5.55$^{cde;fg}$
Week 6	545.00 ± 50.32$^{ab;fg}$	540.50 ± 65.23$^{ab;fg}$	70.50 ± 4.38$^{cde;fg}$	87.50 ± 2.22$^{cde;fg}$	85.00 ± 4.78$^{cde;fg}$

Values are presented as mean ± SD of triplicate determination (N=3). Mean values with same superscript letters are not statistically significant at P ≤ 0.05 down the groups. Superscripts a, b, c, d, e show difference among groups while f, g shows difference among days.

3.1.1. Effects of P. pellucida ethanol leaf extract on lipid profile and Hormone Levels (CRP, PSA, and testosterone)

Tables 3 and 4 show the effects of ethanol extract of *P. pellucida* leaf on lipid profile of Benzo[a]pyrene-Induced Wistar Rats. No significant ($p \leq 0.05$) changes in the activity of the lipid profile parameters were observed in the negative control group and olive oil group at weeks 4 and 6. Results of statistical analyses indicate that benzo[a]pyrene had little to no impact on the lipid profile over a short period of time. However, there were observable changes in HDL. Prostate Specific Antigen (PSA) showed varying levels in groups I and IV rats. At week 6, groups I, IV and V showed non-significant differences in testosterone, PSA and CRP.

3.1.2. Effects of P. pellucida ethanol leaf extract on sperm quality parameters

Tables 5a & b show the effects of ethanol extract of *P. pellucida* leaf on sperm quality parameters in B[a]P-induced Wistar rats. The plant extracts improved sperm count, viability, motility, and morphology as observed at weeks 4 and 6. Changes in semen volume were observed in all groups; groups IV and V rats (curative and prophylactic groups) showed significant ($p \leq 0.05$) elevation in semen volume when compared to the positive control group. Group III showed a significant decrease in semen volume when compared to the prophylactic and curative group. There were significant ($p \leq 0.05$) decreases in sperm viability in group III throughout the experimental duration.

There was significant decrease in % fast progressive mobility in group III rats in comparison with the control groups, and groups IV & V. Meanwhile, % slow progressive mobility had an opposite trend as group III recorded a significant ($p \leq 0.05$) elevation in % slow progressive mobility in comparison with other groups while curative and prophylactic group (group IV and V) recorded non-significant ($p \geq 0.05$) increase in % slow progressive mobility in comparison with curative group.

In the present study, results for lipid profile parameters indicate that B[a]P treatment caused significant increases in Total cholesterol (CHO), and triglycerides (TG) as compared to rats in the negative control group and *P. pellucida* treated groups. Also, there were non-significant elevation ($p \geq 0.05$) in very low density lipoproteins cholesterol (VLDL-C) and high density lipoproteins cholesterol (HDL-C) when compared to the negative control group and *P. pellucida* treated group, which is an indication of risk of cardiovascular disease, stroke, including obesity and metabolic syndrome. Administration of the plant extract resulted in significant reduction ($p \leq 0.05$) in level of Total cholesterol, triglycerides (TG) and, Low Density Lipoproteins Cholesterols (LDL-C) when compared with the positive control group. The decrease in total cholesterol, low density lipoproteins cholesterols (LDL-C) and an increase in high-density lipoproteins cholesterols (HDL-C) in *P. pellucida* treated groups may be due to inhibition of hepatic cholesterol biosynthesis, increased fecal bile acid secretion, stimulation of receptor-mediated catabolism of LDL-cholesterol, or increase in uptake of LDL-cholesterol from the blood by the liver. This corroborated previous findings by Hamzah *et al*. [35] who reported that *P. pellucida* modulates hyperglycemia, oxidative stress and dyslipidemia, and suggested that these activities may be due to the presence of active phytochemicals which has lipid lowering properties.

B[*a*]P is known to have xenoestrogenic action and is currently considered to be an endocrine disruptor [36]. Chronic exposure to B[*a*]P results in a decrease in testosterone production due to disturbances in the steroidogenic machinery of Leydig cells. In mammalian male reproduction, testosterone produced by Leydig cells in response to LH, plays a pivotal role in the initiation and maintenance of spermatogenesis by affecting Sertoli cell androgen receptors. Thus, an abnormal reduction in serum or intratesticular fluid testosterone levels can cause testicular atrophy, which is accompanied by a decrease in the number of germ cells and, ultimately, azoospermia [37].

Spermatogenesis is a controlled process during which a mature sperm is produced from sperm stem cells in three major stages, the mitotic stage, meiotic stage and the maturation stage. Germ-cells undergo mutation during mitotic and meiotic divisions [38]. Findings in this study indicate that B[a]P exposure to experimental animals resulted in depletion of testicular and epididymal functions in animals [39]. According to Zenzes *et al*. [40], escalation of sperm hyperactivity occurs with an increase in the concentration of B[a]P due to premature capacitation. Reddy *et al*. [41] assessed the reproductivity toxicity of B[a]P in adult male Wistar rat and concluded that chronic exposure to B[a]P alters steroidogenesis and spermatogenesis leading to depleted fertility in adult male rats. In this present study, B[a]P treatment caused acute and significant ($p \leq 0.05$) decrease in % semen volume, % sperm viability and % normal sperm morphology. This finding is similar to previous report by Jorge *et al*. [42] and Bukowska *et al*. [43] who both noted that exposure to B[a]P caused low sperm count, and significant decreases in semen volume and sperm viability. The report also noted that B[a]P exposure is capable of causing increase in % dead sperm, slow progressive mobility and abnormal sperm morphology. The prophylactic and curative group recorded a reverse situation, showing improvement in % sperm volume, % sperm viability, fast progressive mobility and % sperm count. Summarily, these findings indicate that B[a]P interferes with sperm quality by altering spermatogenesis. Interestingly however, *P. pellucida* was able to ameliorate the detrimental effects of B[a]P on lipid profile and male fertility indices.

4. Conclusion

Overall, findings from the present study have shown that *P. pellucida* leaves exhibit fertility and cardiovascular amelorative potentials. This is evidenced by the leaf extract's improvement in biomarkers of fertility, as well as cardiovascular indices. The potentials demonstrated are reflective of the wide array of phytochemicals reported to be present in the plant.

Compliance with ethical standards

Statement of ethical approval

All authors hereby declare that "Principles of Laboratory Animal Care" (NIH Publication no. 85- 23, revised 1985) were followed. All experiments were examined and approved by the appropriate ethics committee

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