Protective Effects of Ethanol Leaf Extract of *Peperomia Pellucida* (L.) Kunth on Benzo-a-Pyrene-Induced Toxicity in Wistar Rats

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ABSTRACT

This study evaluated the efficacy of *Peperomia pellucida* (L.) Kunth ethanol leaf extract against PAH-induced organ damage and reproductive dysfunction in adult Wistar rats. Thirty-five (35) rats weighing 150–200g, randomly divided into five (5) groups of seven (7) rats each, were used in this study. Fresh leaves of *Peperomia pellucida* (L.) Kunth were air-dried, ground into fine powder and used in the preparation of an ethanol extract. Toxicity and organ damage was induced using Benzo[a]pyrene (B[a]P). Group I received normal rat feeds and distilled water only for 6 weeks. Group II received 1 ml/kg b.w. of olive oil daily from week 1 to week 3 (orally). Group III 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 3 (orally). Groups IV and V served as curative prophylactic groups respectively. At Weeks 4 and 6, the rats were fasted for 24 h prior to sacrificing/sample collection and then mildly anesthetized with chloroform and blood samples were collected for biochemical analysis. B[a]P induced toxicity in the experimental animals as indicated by significant ($P<0.05$) increase in plasma AST, ALT & ALP activities in the B[a]P-group at weeks 4 and 6. There was significant ($P<0.05$) increase in urea in the B[a]P-induced group, compared to the feeds-only control group which recorded 5.82 ± 1.11 and 5.80 ± 1.27 (µmol/L) at weeks 4 and 6 respectively. Administration of the plant extract led to significant increase in the number of white blood cells (WBC) in the curative group as compared to the B[a]P group (Group III). There was significant increase in the activities of antioxidant enzymes GSH, SOD, MDA and CAT in both curative and prophylactic groups at week 4 and 6. Data obtained from this study suggest that *P. pellucida* leaves exhibit promising hepatoprotective, fertility and antioxidant potency, and consequently has ameliorative effects against Benzo[a]pyrene-induced toxicity and organ damage in Wistar rats.

Keywords: *Peperomia pellucida*; Benzo[a]pyrene; Organ damage; leaf extract; Protective effects

INTRODUCTION

Rapid industrialization and urbanization have resulted in numerous anthropogenic activities that dump various pollutants in the environment, including polycyclic aromatic hydrocarbons (PAHs) [1]. PAHs comprise over 200 organic compounds containing two or more fused aromatic rings [2]. According to the number of aromatic rings, they can be classified as light (2–3 rings) or heavy (4–6 rings) compounds. The inherent properties of PAHs such as heterocyclic aromatic ring structures, hydrophobicity, and thermo stability have made them recalcitrant and highly persistent in the environment. PAH pollutants have been determined to be highly toxic, mutagenic, carcinogenic, teratogenic, and immunotoxicogenic to various life forms [3].

Humans are exposed to PAHs through dietary and non-dietary sources (e.g., inhalation and skin contact). Among these, dietary sources represent the major exposure route. Over 70% of the PAHs exposure of non-smokers is associated with food consumption [4]. Food contamination by PAHs can originate from environmental pollution as well as during food preparation and processing. When contaminated vegetation is used to feed livestock, PAHs start to accumulate in products of animal origin and their derivatives. PAHs exist in several categories of food and drinks as a complex mixture comprising light and heavy compounds [3]. Conversely, PAHs can be formed during food processing, such as smoking or drying—especially when the

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fuel is only partially combusted [6], as well as during preparations involving high temperatures and/or open flames, such as grilling, toasting, roasting, and frying [3], [5]. Industrial, natural, and domestic processes have increased the general population's susceptibility to carcinogens exposure, including PAHs [7]. Therefore, the presence of these organic compounds in foods is concerning and requires continuous monitoring [3].

The European Commission has identified four major PAHs (PAH4) in foods; benzo[a]anthracene (B[a]A), chrysene (Chr), benzo[b]fluoranthene (B[b]F), and benzo[a]pyrene (B[a]P) [8]. Benzo[a]pyrene is the main representative of polycyclic aromatic hydrocarbons (PAHs), and has been repeatedly found in the air, surface water, soil, and sediments. It is present in cigarette smoke as well as in food products, especially when smoked and grilled [9]. B[a]P is of serious concern particularly because of its genotoxic activity following the direct intercalation in the DNA, causing structural disruptions that lead to mutations [10].

Peperomia pellucida (L.) Kunth is an herbaceous plant belonging to *Piperaceae* family. 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 [11]. Pharmacological study reports reveal that this plant is antipyretic, analgesic, anti-inflammatory, antimicrobial, refrigerant, antioxidant, anti-hyperglycemia, anti-hyperuricemia, has burn healing and depressant effect, gastro protective, hypotensive, cytotoxic, anti-sickling cell, lipase inhibitory, fibrinolytic and thrombolytic, antidiarrheal, and has anti-osteoporotic activities. In addition, there are also claims that the plant also has cosmetic benefits [12], [13]. Phytochemical studies reports showed that plant extract of *Peperomia pellucida* (L.) Kunth is rich in mineral content such as sodium, potassium, calcium, zinc, iron, manganese, lead and phosphorus [14]. In addition to minerals, this plant also known to contain many bioactive substances such as stigma sterol [15]; -amino acid,α, alkaloid, glycoside, reducing sugar, flavonoid, tannin, steroid, terpenoid, neutral compound, phenolic compound and starch [16]; β-caryophyllene, αhumulene, epi-α-bisabolol, sabinene, crypt one and caryophyllene oxide [17].

Studies on the medicinal properties of *Peperomia pellucida* (L.) Kunth other than those already believed and practiced by local communities are still very limited. Considering the reproductive and organ toxicity of benzo[a]pyrene after oral or inhalation exposure, the focus of the study is to ascertain the potency of *Peperomia pellucida* (L.) Kunth leaves against toxicological effects caused by Benzo[a]pyrene (B[a]P) which is the main representative of polycyclic aromatic hydrocarbons (PAHs), found in the air, surface water, soil, sediments and food products.

MATERIALS AND METHODS

Experimental Animals

Wistar rats weighing 150-200g were obtained from the Animal House of the Department of Biochemistry, University of Port Harcourt, Nigeria. The rats were acclimatized in a temperature-controlled animal house (25 ± 2 °C) under 12 h of light and 12 h of darkness for 14 days before the experiment, and then assigned randomly into five (5) groups of seven (7) rats each. The rats were housed in standard iron cages and given ad libitum access to standard pelletized commercial rat feed and distilled water throughout the experimental period. Animal handling followed the procedures of the National Institute of Health Guide on the use of Animals for Experiments.

Chemicals

The reagents used for the experiments 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 Enzyme Assay Kits).

Preparation of Ethanol Leaf Extract of Peperomia Pellucida (L.) Kunth

Fresh leaves of *Peperomia pellucida* (L.) Kunth plant were obtained from Aluu in Port Harcourt, Rivers State, Nigeria. The plant was identified and authenticated by a Plant Technologist in the Department of Plant Science and Biotechnology, University of Port Harcourt, Nigeria. The freshly collected leaves of *Peperomia pellucida*

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(L.) Kunth plant were thoroughly washed with tap water followed by distilled water. The leaves were air-dried in the shade at 33°C ± 2°C and ground into a fine powder using mechanical grinder. *P. pellucida* (L.) Kunth aqueous extract was prepared according to a previously reported method [18]. Dry powder (50 g) 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. They were labelled appropriately and stored in plastic bags at -20 °C for the experiment.

Experimental Grouping

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

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 week 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 week 6 (orally).

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

In designing the Experimental Groups, LD_{50} value of 4000mg/kg b. w. was considered for *Peperomia pellucida* leaf extract as reported in a previous study [19]. Doses administered for Benzo[a]pyrene were guided by a previous research [20] and the no-observed-adverse-effect level (NOAEL) & lowest observed adverse effect level (LOAEL) values shown in Table 3.1, sourced from the Danish Environmental Protection Agency [21]. Olive oil was administered following the dosage administered by [22].

Table 1. Summary of NOAELs/LOAELs from short-term toxicity tests of some PAH compounds following oral administration (gavage) [21]

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

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The experiment lasted for 6 weeks. At weeks 4 and 6, the rats were fasted for 24 h prior to sampling and then mildly anesthetized with chloroform. 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 assay for liver function, kidney function, hematological parameters and activity of oxidative enzymes.

Biochemical Analysis

Hepatotoxic Biomarkers

Plasma L - alanine aminotransferase (ALT) and L - aspartate aminotransferase (AST) activities were determined using the method described by Reitman and Frankel [23] while Alkaline phosphatase (ALP) activity was measured using the colorimetric method using phenolphthalein monophosphate as substrate [24]. Bilirubin was determined using the method described by Jendrasik & Grof [25]. Serum Total Protein level was measured using the Biuret Method [26]. Determination of serum Albumin concentration was by the Biromoeresol Green Method described by Rodkey [27].

Nephrotoxic Biomarkers

Plasma sodium determination followed the precipitation method described by Henry [28]. Serum Potassium ion concentration was measured using the Tetraphenylborate method described by Tietz [29]. Urea Estimation Blood urea concentrations were determined using Berthelot's reaction as described by Kaplan and Teng [30]. Creatinine Estimation Assay for creatinine was carried out using the Reflotron Dry Chemistry Analyzer as described by Estridge *et al.* [31].

Determination of Hematological Parameters

Blood samples used for analysis of hematological parameters were collected in EDTA bottles and were analyzed using a haematology analyzer (Mindray Auto Hematology Analyzer, BC-5200, USA) following the manufacturer's instructions. Parameters analyzed were red blood cell count (RBC), packed cell volume (PCV), white blood cell count (WBC) and haemoglobin (Hb).

Antioxidant Assays

Determination of malondialdehyde levels was carried out using the lipid peroxide assay method described by Varshney and Kale [32]. Superoxide dismutase (SOD) activity was assayed using the method described by Fridorich [33] and as contained in the Randox commercial kit. Catalase activity was assayed using the method of Aebi [34]. The concentration of glutathione was determined according to the method of Habig *et al*. [35].

Statistical Analysis

All values were expressed as mean ± SD and then subjected to analysis of variance (ANOVA) using the Statistical Package for Social Sciences (SPSS) version 17.0 (SPSS Inc., Chicago Illinois). Statistical significance was considered at P=0.05.

RESULTS AND DISCUSSION

Table 2. Effects of Ethanol Extract of *P. pellucida* leaf on Liver Function Parameters in Benzo[a]pyrene-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)**
ALT (µ/L)					
Week 4	$20.07 \pm 0.08^{ab;fg}$	$21.12 \pm 3.56^{ab;fg}$	$38.30 \pm 3.12^{cd;fg}$	$34.44 \pm 4.22^{cde;f}$	$30.71 \pm 3.65^{de;fg}$
Week 6	$20.25 \pm 0.87^{abd;fg}$	$20.34 \pm 2.06^{abd;fg}$	$39.33 \pm 4.55^{c;fg}$	$27.00 \pm 5.12^{abde;g}$	$28.21 \pm 4.56^{de;fg}$

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AST (µ/L)					
Week 4	40.44 ± 0.77$^{ab;fg}$	43.27 ± 4.25$^{ab;fg}$	70.78 ± 7.44$^{cd;fg}$	65.05 ± 6.88$^{cde;f}$	55.86 ± 5.22$^{de;fg}$
Week 6	40.15 ± 0.17$^{ab;fg}$	37.53 ± 3.39$^{ab;fg}$	68.91 ± 3.98$^{c;fg}$	51.45 ± 7.77$^{de;g}$	52.77 ± 3.98$^{de;fg}$
ALP (µ/L)					
Week 4	31.61 ± 1.08$^{ab;fg}$	31.11 ± 4.99$^{ab;fg}$	64.62 ± 4.77$^{cd;fg}$	57.51 ± 4.56$^{cd;f}$	44.00 ± 5.11$^{e;fg}$
Week 6	30.81 ± 0.72$^{ab;fg}$	30.25 ± 2.98$^{ab;fg}$	65.05 ± 5.88$^{c;fg}$	41.78 ± 4.21$^{de;g}$	40.40 ± 3.25$^{de;fg}$
TP (g/L)					
Week 4	66.58 ± 1.16$^{abe;fg}$	68.56 ± 9.80$^{ab;fg}$	35.32 ± 6.23$^{cd;fg}$	38.50 ± 3.98$^{cd;f}$	56.00 ± 6.33$^{ae;fg}$
Week 6	66.99 ± 0.01$^{abe;fg}$	62.29 ± 4.81$^{abde;fg}$	33.70 ± 3.98$^{c;fg}$	58.51 ± 4.66$^{bde;g}$	60.71 ± 4.99$^{abde;fg}$
Albumin (g/L)					
Week 4	44.97 ± 0.94$^{abe;fg}$	46.11 ± 0.18$^{abe;fg}$	25.80 ± 5.65$^{cd;fg}$	28.62 ± 8.11$^{cd;fg}$	41.77 ± 6.22$^{abe;fg}$
Week 6	46.11 ± 1.17$^{abe;fg}$	51.12 ± 6.00$^{abe;fg}$	24.05 ± 4.22$^{c;fg}$	37.00 ± 6.27d$^{e;fg}$	43.44 ± 4.95$^{abde;fg}$
TB (µmol/L)					
Week 4	4.58 ± 0.44$^{abcde;fg}$	4.88 ± 3.00$^{abcde;fg}$	8.40 ± 1.88$^{abcde;fg}$	8.15 ± 5.78$^{abcde;fg}$	7.65 ± 5.55$^{abcde;fg}$
Week 6	4.86 ± 0.20$^{abcde;fg}$	4.44 ± 1.20$^{abde;fg}$	9.33 ± 3.67$^{acde;fg}$	6.82 ± 2.21$^{abcde;fg}$	7.00 ± 3.88$^{abcde;fg}$
CB (µmol/L)					
Week 4	2.44 ± 0.25$^{abde;fg}$	2.98 ± 0.80$^{abcde;fg}$	5.15 ± 1.98$^{bcde;fg}$	4.60 ± 1.22$^{abcde;fg}$	3.90 ± 1.66$^{abcde;fg}$
Week 6	2.44 ± 0.07$^{abde;fg}$	2.91 ± 0.30$^{abde;fg}$	5.20 ± 1.06$^{c;fg}$	3.00 ± 0.98$^{abde;fg}$	3.00 ± 1.18$^{abde;fg}$

Values are presented as mean ± SD of triplicate determination (N=3). Mean values with same superscript letters are not statistically significant at $P \leq 0.05$ across the groups. Superscripts a, b, c, d, e show difference among groups while f, g shows difference among days. **ALT**=Alanine Aminotransferase, **AST**=Aspartate aminotransferase, **ALP**= Alkaline Phosphatase, **TP** - T-Protein, **ALB** – Albumin, **TB** – Total Bilirubin, **CB** – Conjugated Bilirubin.

Table 3. Effects of Ethanol Extract of *P. pellucida* leaf on Kidney Function Parameters in Benzo[a]pyrene-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)**
Urea (µmol/L)					
Week 4	5.82 ± 1.11$^{abe;fg}$	5.61 ± 1.42$^{abe;fg}$	10.07 ± 3.25$^{cde;fg}$	9.78 ± 2.22$^{cde;f}$	7.07 ± 1.77^{abcde,fg}
Week 6	5.80 ± 1.27$^{abde;fg}$	5.64 ± 0.89$^{abde;fg}$	10.12 ± 2.32$^{c;fg}$	5.78 ± 1.30$^{abde;g}$	5.70 ± 1.21$^{abde;fg}$
Creatinine (µmol/L)					
Week 4	118.80 ± 12.85$^{ab;fg}$	110.45 ± 14.44$^{ab;fg}$	168.00 ± 20.31$^{cde;fg}$	151.55 ± 10.22$^{cde;f}$	150.31 ± 16.77^{cde,f}
Week 6	120.00 ±18.44$^{abde;fg}$	111.21 ±17.21$^{abde;fg}$	165.05 ± 15.48$^{c;fg}$	115.88 ± 14.32$^{abde;g}$	120.60 ± 10.46$^{abde;g}$
K+ (mmol/L)					
Week 4	4.31 ± 0.98$^{abde;fg}$	4.55 ± 1.11$^{abcde;fg}$	7.25 ± 1.77$^{bcde;fg}$	6.85 ± 2.12^{abcde,fg}	6.76 ± 1.31^{abcde,fg}
Week 6	4.22 ± 1.55$^{abde;fg}$	3.98 ± 0.59$^{abde;fg}$	7.20 ± 1.93$^{cde;fg}$	5.33 ± 1.09$^{abcde;fg}$	5.76 ± 1.05$^{abcde;fg}$
Na+ (mmol/L)					
Week 4	112.50 ± 14.78$^{abe;fg}$	112.67 ± 10.10$^{abe;fg}$	172.11 ± 17.69$^{cd;fg}$	150.14 ± 11.66^{cd,f}	123.51 ± 14.41^{abe,fg}
Week 6	111.71 ± 21.33$^{abde;fg}$	113.00 ± 13.22$^{abde;fg}$	170.00 ± 23.23$^{c;fg}$	108.98 ± 14.93$^{abde;g}$	123.00 ± 15.22$^{abde;fg}$

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

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Table 4. Effects of Ethanol Extract of *P. pellucida* leaf on Hematological Parameters in Benzo[a]pyrene-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)
WBC ($x10^3$ /mm^3)					
Week 4	$28.76 \pm 4.78^{ab;fg}$	$25.28 \pm 5.98^{abde;fg}$	$14.87 \pm 4.77^{cde;fg}$	$18.43 \pm 1.55^{bcde;fg}$	$18.56 \pm 1.66^{bcde;fg}$
Week 6	$29.00 \pm 5.21^{abde;fg}$	$25.00 \pm 6.21^{abde;fg}$	$14.80 \pm 6.21^{cd;fg}$	$22.87 \pm 3.31^{abde;fg}$	$25.00 \pm 1.88^{abde;fg}$
Platelet (10^9/L)					
Week 4	$456.78 \pm 25.98^{abde;fg}$	$444.21 \pm 30.55^{abde;fg}$	$562.11 \pm 45.98^{c;fg}$	$477.00 \pm 16.56^{abde;fg}$	$481.20 \pm 30.30^{abde;fg}$
Week 6	$456.00 \pm 31.23^{abde;fg}$	$443.00 \pm 35.11^{abde;fg}$	$555.32 \pm 50.11^{c;fg}$	$440.00 \pm 21.87^{abde;fg}$	$450.38 \pm 20.41^{abde;fg}$
Hemoglobin (g/dl)					
Week 4	$15.00 \pm 4.22^{abcde;fg}$	$15.05 \pm 3.78^{abcde;fg}$	$10.91 \pm 2.21^{abcde;fg}$	$12.33 \pm 1.56^{abcde;fg}$	$13.00 \pm 1.67^{abcde;fg}$
Week 6	$15.00 \pm 2.79^{abcde;fg}$	$14.50 \pm 3.91^{abcde;fg}$	$11.15 \pm 3.41^{abcde;fg}$	$12.00 \pm 2.41^{abcde;fg}$	$12.00 \pm 1.88^{abcde;fg}$
PCV (%)					
Week 4	$35.05 \pm 5.32^{abcde;fg}$	$36.00 \pm 4.21^{abcde;fg}$	$40.50 \pm 3.12^{abcde;fg}$	$37.17 \pm 5.89^{abcde;fg}$	$39.00 \pm 3.41^{abcde;fg}$
Week 6	$34.21 \pm 6.21^{abcde;fg}$	$37.82 \pm 7.22^{abcde;fg}$	$41.00 \pm 3.41^{abcde;fg}$	$35.35 \pm 1.22^{abcde;fg}$	$37.00 \pm 1.22^{abcde;fg}$
Lymphocytes (%)					
Week 4	$84.16 \pm 6.25^{abe;fg}$	$82.88 \pm 6.67^{abe;fg}$	$25.33 \pm 5.36^{c;fg}$	$68.18 \pm 2.43^{d;fg}$	$81.5 \pm 4.38^{abe;fg}$
Week 6	$80.5 \pm 8.32^{abe;fg}$	$82.00 \pm 5.89^{abe;fg}$	$26.91 \pm 6.21^{c;fg}$	$60.65 \pm 2.22^{d;fg}$	$82.00 \pm 2.89^{abe;fg}$
Week 4	$3.83 \pm 0.78^{ab;fg}$	$3.71 \pm 1.10^{ab;fg}$	$28 \pm 2.43^{c;fg}$	$12.71 \pm 1.15^{de;fg}$	$15.44 \pm 3.33^{de;fg}$
Week 6	$2.88 \pm 0.51^{ab;fg}$	$4.00 \pm 1.29^{ab;fg}$	$28.00 \pm 2.89^{c;fg}$	$12.00 \pm 2.11^{de;fg}$	$14.00 \pm 2.21^{de;fg}$

Values are presented as mean ± SD of triplicate determination (N=3). Mean values with same superscript letters are not statistically significant at $P \leq 0.05$ across the groups. Superscripts a, b, c, d, e show difference among groups while f, g shows difference among days. **WBC** – White blood cell, **PCV** - Packed Cell Volume,

Table 5. Effects of Ethanol Extract of *P. pellucida* leaf on Antioxidant Enzymes in Benzo[a]pyrene-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)
SOD (µ/ml)					
Week 4	$0.65 \pm 0.05^{abcde;fg}$	$0.66 \pm 0.06^{abcde;fg}$	$0.58 \pm 0.09^{abcde;fg}$	$0.61 \pm 0.07^{abcde;fg}$	$0.75 \pm 0.34^{abcde;fg}$
Week 6	$0.71 \pm 0.08^{abcde;fg}$	$0.90 \pm 0.04^{abde;fg}$	$0.58 \pm 0.06^{ac;fg}$	$0.82 \pm 0.03^{abde;fg}$	$0.91 \pm 0.23^{abde;fg}$
MDA (µmol/ml)					
Week 4	$0.15 \pm 0.00^{ab;fg}$	$0.13 \pm 0.02^{ab;fg}$	$0.28 \pm 0.03^{c;f}$	0.42 ± 0.01^{de}	$0.47 \pm 0.11^{de;fg}$
Week 6	$0.30 \pm 0.03^{abde;fg}$	$0.13 \pm 0.03^{abde;fg}$	$9.22 \pm 1.23^{c;g}$	$0.55 \pm 0.04^{abde;fg}$	$0.51 \pm 0.00^{abde;fg}$
GSH (µ/ml)					
Week 4	$2.41 \pm 0.29^{ab;fg}$	$2.33 \pm 0.40^{ab;fg}$	$1.44 \pm 0.41^{c;fg}$	$3.44 \pm 0.22^{d;f}$	$4.48 \pm 0.41^{e;fg}$
Week 6	$2.33 \pm 0.56^{ab;fg}$	$2.33 \pm 0.35^{ab;fg}$	$1.40 \pm 0.34^{c;fg}$	$4.33 \pm 0.41^{de;g}$	$4.33 \pm 0.34^{de;fg}$
CAT (µ/g)					
Week 4	$16.45 \pm 5.10^{ab;fg}$	$12.10 \pm 1.56^{abcde;fg}$	$7.60 \pm 1.36^{bcde;fg}$	$8.42 \pm 1.10^{bcde;fg}$	$9.93 \pm 1.31^{bcde;fg}$
Week 6	$13.00 \pm 1.23^{abe;fg}$	$13.00 \pm 2.11^{abe;fg}$	$7.70 \pm 1.47^{cde;fg}$	$9.11 \pm 1.85^{cde;fg}$	$10.05 \pm 2.31^{abcde;fg}$

Values are presented as mean ± SD of triplicate determination (N=3). Mean values with same superscript letters are not statistically significant at $P \leq 0.05$ across the groups. Superscripts a, b, c, d, e show difference among groups while f, g shows difference among days. **SOD** - Superoxide Dismutase, **MDA** – Malondialdehyde, **GSH** – Glutathione, **CAT** – Catalase.

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Effects of P. pellucida (L.) Kunth ethanol leaf extract on liver function parameters

Table 2 shows serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) activities following administration of *P. pellucida* and/or Benzo[a]pyrene (B[a]P). B[a]P induced toxicity in the experimental animals as indicated by significant ($P<0.05$) increase in plasma AST, ALT & ALP activities in the B[a]P-group at weeks 4 and 6. When compared to Group I, II (Control groups), Group IV (curative group), and Group V (prophylactic group), activities of AST and ALP in Group III rats were significantly ($p\leq0.05$) elevated. The Benzo[a]pyrene group had significantly higher enzymatic activity at week 4 and 6 compared to the other groups, while group V (prophylactic group) had decreased enzyme activities. This suggests that *P. pellucida* extract will have better activity over a longer period of time.

While group III showed a significant ($p\leq0.05$) increase in the activities of total bilirubin and conjugated bilirubin when compared to other groups, total protein and albumin levels were significantly ($p\leq0.05$) decreased in group III, while there was no significant difference ($p\leq0.05$) in total bilirubin and conjugated bilirubin when group I and II were compared to group IV and V group. Groups I and II differed considerably ($p\leq0.05$) from one another, but their differences from group IV were non-significantly higher because the curative group's activities were more similar to those of groups I and II. Group IV and V (both experimental groups) recorded higher albumin and total protein levels.

Effects of P. pellucida (L.) Kunth ethanol leaf extract on kidney function parameters

Tables 3 shows the effects of ethanol leaf extract of *P. pellucida* (L.) Kunth on kidney function indices in B[a]P-Induced Wistar Rats. There was significant ($p<0.05$) increase in urea in the B[a]P-induced group, compared to the control group which recorded 5.82 ± 1.11 and 5.80 ± 1.27 (μmol/L) at weeks 4 and 6 respectively. When compared statistically to groups I, II, and IV, urea levels in group III increased significantly ($p\leq0.05$), but group IV differed from group III at the end of the 14-day treatment period. In contrast, creatinine levels in group III increased significantly ($p\leq0.05$) when compared to groups I, II, IV, and V at the end of the 28-day treatment period; at the end of the 14-day treatment period, group IV and V recorded non-significant differences in comparison to group III. Similar trends were observed in the activities of potassium and sodium, where group III potassium and sodium activities were significantly ($p\leq0.05$) higher at 28 days of treatment than group IV and V at the same time, as well as in control groups I and II, at the same time.

Effects of P. pellucida (L.) Kunth ethanol leaf extract on hematological parameters

Table 4 shows the effects of ethanol extract of *P. pellucida* (L.) leaves on hematological parameters of Benzo[a]pyrene-Induced Toxicity in Wistar Rats. Administration of the plant extract led to a significant increase in the number of white blood cells (WBC) in the curative group as compared to the B[a]P group (Group III). There were significant ($p \leq 0.05$) reduction in WBC, Hemoglobin and lymphocytes in group III as compared to other groups in the study. Groups IV and V had non-significant difference at week 6. Percentage lymphocytes had non-significant changes in group V in both treatment groups compared to the control groups (I and II) while Group III recorded significant reduction in the activities of lymphocytes when compared to the control groups.

Effects of P. pellucida (L.) Kunth ethanol leaf extract on antioxidant enzymes

Table 5 shows the effects of ethanol extract of *P. pellucida* leaf on antioxidant enzymes in Benzo[a]pyrene-Induced Wistar Rats**.** There were significant increases in the activities of antioxidant enzymes GSH, SOD, MDA and CAT in both curative and prophylactic groups at week 4 and 6. There were non-significant ($p\leq0.05$) reduction in the activities of SOD (superoxide dismutase) in group III when compared to group I, II, IV and V. Significant increases in the activities of SOD were also observed in groups I, II, IV and V when compared to group II at week 6 of treatment. MDA showed non-significant differences in group IV and V but were statistically different when compared to III. GSH activities recorded significant decreases in group III when compared to other groups.

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Results obtained in this present study showed that animals induced with toxicity using Benzo[a]pyrene showed elevated liver enzyme activities. The liver enzymes - Alanine transaminase (ALT), Aspartate transaminase (AST) and Alkaline phosphatase (ALP) are important liver markers used to monitor the functions of the liver [36]. This study observed a significant ($p< 0.05$) elevation in ALT, AST, ALP, TB and CB in the B[a]P group at weeks 4 and 6. The prophylactic group and curative treated group recorded a significant reduction in the activities of these enzymes when compared to the B[a]P treated group. B[a]P caused injury in the hepatic tissue and hepatocytes. Pre and post treatment with *P. pellucida* were effective in counteracting B[a]P-induced hepatic damage, Oxidative stress and cellular necrosis. The results indicate that *P. pellucida* possesses prophylactic, as well as therapeutic or curative potentials against B[a]P induced hepatic injury. The pre-administration of *P. pellucida* (prophylactic) boosted the immunity of the experimental animal's system, though post-treatment administration had more positive effects on the experimental animals than the pre-treatment group. Results for albumin and total protein in the B[a]P group indicated hepatotoxicity caused by B[a]P and decreased the ability of the liver to synthesize proteins, though this was arrested to an extent in prophylactic group and as such *P. pellucida* acted as a prophylactic agent against the detrimental effect of B[a]P on the liver. Also, there were significant increases in total protein and albumin in the post-treatment with *P. pellucida*. This is indicative that *P. pellucida* was able to reverse the hepatotoxicity effects of B[a]P. Significant elevation in Total Bilirubin (TB) and Conjugated Bilirubin (CB) was observed in the B[a]P group. However, *P. pellucida* was able to reverse this marked elevation of CB but had little or no effect on TB. This result indicates a high degree of hepatotoxicity protection potential of *P. pellucida* on experimental animals against B[a]P induced toxicity. This result is in agreement with Munkong *et al.* [37] and Huang *et al.* [38] who reported in separate studies that the administration of plant extracts on experimental animals were able to act as prophylactic and therapeutic agents against B[a]P induced liver damage and elevation of ALT, AST, ALP, TB, CB also the reduction in Albumin and Total protein.

The present investigation revealed increases in plasma creatinine, sodium, potassium and urea levels in the B[a]P-treated rats, indicating renal dysfunction in the animals. Elevated plasma urea signifies decreased reabsorption at the renal epithelium whereas high plasma creatinine revealed impairment in the renal functions while sodium and potassium will alter sodium balance, causing the kidneys to have reduced function and remove less water resulting in higher blood pressure, mostly for glomerular filtration rate in the B[a]P-treated rats [39]. Interestingly, pre-treatment and post treatment with *P. pellucida* remarkably reversed the B[a]P-mediated increase in the plasma levels of renal functional indices (sodium, potassium, urea and creatinine). This correlates with the works of Xu *et al*. [40] and Narayanamoorthi *et al*. *[13]*. The restoration of these biomarkers indicates a protective and therapeutic effect of *P. pellucida* against renal toxicity resulting from B[a]P exposure.

The study also investigated effects of *P. pellucida* leaf extract on Benzo (a) pyrene- induced hematological changes in Wistar rats. Among different parameters that were assayed were hemoglobin, white (WBC), Platelet, packed cell volume (PCV) and Lymphocytes. Hemoglobin is an iron containing protein pigment present in erythrocytes that imparts characteristic red color to blood. Its major role is in the transport of oxygen throughout the body and its deficiency may result in pathological condition called anemia; White Blood Cell (WBC) count is the total number of leukocytes in the blood per unit volume. High WBC count usually means that the body is fighting an infection whereas a very low WBC count can be caused by problems with the bone marrow. The later condition is called cytopenia or leukopenia which means that organism is less able to fight off infections. Lymphocytes are non-motile and non-phagocytic cells. Anatomically, an important distinguishing feature of lymphocytes is their large nuclei with scanty cytoplasm. One group of lymphocytes, B-lymphocytes, are precursor of plasma cells that produce antibodies. Other lymphocytes, T-lymphocytes, are involved in immune reactions, such as graft rejection and delayed hypersensitivity. Observation from this research work indicates that B[a]P caused a significant reduction in the activities of white blood cells, hemoglobin, and lymphocytes this clearly reveals that B[a]P cause destruction of WBC, decrease in Hb concentration and lymphocyte causing a reduction in the capability of blood in the transporting of oxygen and depletion of the volume of oxygen delivered to the tissues which also caused decreased WBC and lymphocytes levels when compared with the control groups, prophylactic and curative groups. The plant extracts under study was able to significantly ($p\leq0.05$) boost the immune system of experimental animals as observed in increase in WBC, Hb, and lymphocytes levels. Also, there were increases in PCV and platelet levels in B[a]P

treated group. It can be inferred that *P. pellucida* cushions detrimental effects of B[a]P. This finding corroborates previous research works [41, 42].

Hematological parameters are routinely monitored during the course of treatment of diseases for assessing the overall wellbeing as well as the effects of the treatment; they are also useful indices that can be employed to assess that toxicity of a plant product, chemicals or environmental toxicants in living system. They also give a clear insight and explanation on the biochemical relationship (interaction) between the blood and functions of chemical plant product in vivo. Hematological abnormalities associated with liver disease and research has shown that exposure of B[a]P causes negative interaction in hematological parameter in living Cells [43].

SOD is known to accelerate the conversion of endogenous cytotoxic superoxide radicals to H_2O_2 whereas CAT acts to eliminate H_2O_2 thereby protecting the cells. Moreover, GSH and GSH-dependent enzymes namely GPx and GST participate in the glutathione redox cycle by converting H_2O_2 and lipid peroxides to non-toxic products [39, 44, 45]. MDA level, a biomarker of lipid peroxidation, is a well-established indicator of oxidative stress in cells and tissues. In the present study, B[a]P-treated rats showed marked diminution in GSH level, CAT, and SOD activities of these antioxidant enzymes with significant ($p< 0.05$) elevation in MDA levels but P. *pellucida* post treatment and pretreatment were able to restore this effect when compared to the controls, these observations clearly indicate a compromised antioxidant defense system, inflammation and a state of oxidative stress in the renal tissues of B[a]P-exposed rats. The present observations are in agreement with the previous reports that B[a]P-exposure induced oxidative stress and inflammation in experimental animals [46, 47].

The increased intra-tubular protein cast indicates nephrotic glomerular dysfunction in the B[a]P-treated rats. Interestingly, *P. pellucida* post-treatment and pre-treatment significantly improved the antioxidant status and ameliorated detrimental effects of B[a]P, thus demonstrating the protective and therapeutic effect of *P. pellucida* in B[a]P-induce toxicity. The ameliorative and therapeutic effects of *P. pellucida* in B[a]P-induced toxicity is attributed to the enhancement antioxidant defense mechanism. Thus, dietary inclusion of *P. pellucida* could exert protective and prophylactic effects against toxicity resulting from B[a]P-exposure.

CONCLUSION

The present study has shown that *P. pellucida P. pellucida* leaves exhibit promising hepatoprotective and antioxidant potency, and consequently has ameliorative effects against Benzo[a]pyrene-induced toxicity and organ damage in wistar rats.

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.

Competing Interests

Authors have declared that no competing interests exist.

REFERENCES

1. Mojiri, A., Zhou, J. L., Ohashi, A., Ozaki, N. & Kindaichi, T. (2019). Comprehensive review of polycyclic aromatic hydrocarbons in water sources, their effects and treatments. Sci Total Environ., 696, 133971.
2. Abdel-Shafy, H. I. & Mansour, M. S. (2016). A review on polycyclic aromatic hydrocarbons: source, environmental impact, effect on human health and remediation. Egypt. J. Pet., 25, 107–123.
3. Purcaro, G., Moret, S. & Conte, L. S. (2013). Overview on polycyclic aromatic hydrocarbons: Occurrence, legislation and innovative determination in foods. Talanta, 105.

4. Rengarajan, T., Rajendran, P., Nandakumar, N., Lokeshkumar, B., Rajendran, P. & Nishigaki, I. (2015). Exposure to polycyclic aromatic hydrocarbons with special focus on cancer. Asian Pac. J. Trop. Biomed., 5, S1691–S2221.
5. Garcia, L. P., Gonçalves, B. L., Panho, G. & Scussel, V. M. (2014). Hidrocarbonetos policíclicos aromáticos em alimentos: Uma revisão. Pubvet, 8.
6. Hamidi, E.N., Hajeb, P., Selamat, J. & Razis, A. F. A. (2016). Polycyclic aromatic hydrocarbons (PAHs) and their bioaccessibility in meat: A tool for assessing human cancer risk. Asian Pac. J. Cancer Prev., 17, 15–23.
7. Sampaio, F., Sequeira, C. & Teixeira, L. (2021). Impact of COVID-19 outbreak on nurses'mental health: a prospective cohort study. Environ. Res., 194, 110620.
8. Sampaio, G. R., Guizellini, G. M., da Silva, S. A., de Almeida, A. P., Pinaffi-Langley, A. C. C., Rogero, M. M., de Camargo, A. C. & Torres, E. A. F. S. (2021). Polycyclic Aromatic Hydrocarbons in Foods: Biological Effects, Legislation, Occurrence, Analytical Methods, and Strategies to Reduce Their Formation. Int J Mol Sci., 22(11):6010.
9. Ujowundu, C. O., Ogbede, J. U., Igwe, K. O., Okwu, G. N. & Agha, N. C. (2014). Quantitative Assessment of Polycyclic Aromatic Hydrocarbons and Heavy Metals in Fish Roasted with Firewood, Waste Tyres and Polyethylene Materials. Biochem Anal Biochem, 4, 162.
10. Malarkey, D. E., Hoenerhoff, M. J. & Maronpot, R. R. (2018). Carcinogenesis. In Fundamentals of Toxicologic Pathology, 3rd ed.; Wallig, M.A., Haschek, W.M., Rousseaux, C.G., Bolon, B., Eds.; Academic Press: San Diego, CA, USA, pp. 83–104.
11. Susilawati, Y., Nugraha, R., Krishnan, J., Muhtadi, A., Sutardjo, S. & Supratman, U. (2017). A New Antidiabetic Compound 8.9- dimethoxy Ellagic Acid from Sasaladaan (PeperomiapellucidaL. Kunth). Research Journal of Pharmaceutical. Biological and Chemical Sciences, 8(1S), 269-274.
12. Kartika, I, Insanu, M., Safitri, D., Putri, C. A. & Adnyana, I. K. (2016). New Update: Traditional Uses. Phytochemical. Pharmacological and Toxicity Review of Peperomia pellucida (L.) Kunth. Pharmacology online, 31, 30-43.
13. Narayanamoorthi, V., Vasantha, K., & Maruthasalam, R. R. A. A. (2015). GC MS determination of bioactive components of Peperomia pellucida (L.) Kunth. Bioscience Discovery, 6(2), 83-88.
14. Ojo, O. O., Ajayi, S. S. & Owolabi, L. O. (2012). Phytochemical screening. Anti-nutrient composition. Proximate analyses and the antimicrobial activities of the aqueous and organic extracts of bark of Rauvolfiavomitoria and leaves of Peperomia pellucida. International Research Journal of Biochemistry and Bioinformatics, 2(6), 127-134.
15. Hartati, S., Angelina, M., Dewiyanti, I. & danMeilawati, L. (2015). Isolation and Characterization Compounds from Hexane and Ethyl Acetate Fractions of Peperomia pellucida L. The Journal of Tropical Life Science, 5(3), 117- 122.
16. Htet Y. M. S. & Khaing M. M. (2016). Botanical Studies and Phytochemical Screening of Peperomia pellucida (L.) Kunth (Thit-YayGyi). Hinthada University Research Journal, 7(1).
17. Lira, P. D. L., Farfán, Y., van Baren, C. M., Bandoni, A. L., Coussio, J. D. & de Abram A. P. (2007). Composition of the essential oil of two Peperomia from Peru: P. Galioides and P. Chalhuapuquiana Rev. Latinoamer. Quím., 35/1, 7-12.
18. Lemhadri A, Zeggwagh NA, Maghrani A, Jouad H, Eddouks M. (2004). Anti-hyperglycaemic activity of the aqueous extract of Driganum vulgare growing wild in Tafilalet region. J. Ethanopharmacol. 90:251–256.
19. Desy, R. W., Fadlina, C. S. & Abdul, M. (2017). Secondary Metabolites Screening and Acute Toxicity Test of Peperomia pellucida (L.) Kunth Methanolic Extracts. International Journal of PharmTech Research, 10(1):31-38.
20. Kolade, O. Y. & Oladiji, T. A. (2019). Protective Effects Of Curcumin Against Benzopyrene Induced Liver Toxicity In Albino Rats. Earth and Environmental Science 210: 012013
21. Danish Environmental Protection Agency (2013). Polyaromatic Hydrocarbons (PAH) Evaluation of health hazards and estimation of a quality criterion in soil Environmental Project No. 1523.
22. Akinlolu, A. A., Ebito, G., Ameen, M., Asogwa, N., Odubela, K., Akindele, R., Bamidele Fagbohunka, B., Ajayi, N., Sobande, I. & Temitope Sikiru, T. (2022). Cadmium Chloride-Induced Skin Ulceration

and Inflammation in Rats: Anti-Inflammatory Potentials of Moringa Oleifera and Musa Sapientium via Nf- Kb/Il-4/1l-10-Mediated Pathway. Anatomy Journal of Africa, 11 (2):2285-2295.
23. Reitman, S. & Frankel, S. (1957). Glutamic – pyruvate transaminase assay by colorimetric method. Am. J. Clin. Path. 28:56.
24. Plummer, D. T. (1978). An introduction to Practical Biochemistry, 3rd edition. McGraw – Hill Book Company, Maidenhead, Berkshire.
25. Jendrassik, L. & Grof, P. (1938). Colorimetric Method of Determination of bilirubin. Biochem Z., 297:81-82
26. Lubran, M. M. (1978). The Measurement of Total Serum Proteins by the Biuret Method. Annals of Clinical and Laboratory Science, 8(2).
27. Rodkey, F. L. (1965). Direct spectrophotometric determination of albumin in human serum. Clin Chem., 11: 478-87.
28. Henry, R. J. (1974). Clinical Chemistry. 2nd edition. Harper & Row, New York.
29. Tietz, N. W. (1976). Fundamentals of Clinical Chemistry, 2nd edition, Saunders, Philadelphia.
30. Kaplan, A. & Teng, L. L. (1982). In Selected Methods of Clinical Chemistry, Vol. 9, Faulkner WR and Meites S Eds., AACC, Washington.
31. Estridge, B. H., Reynolds, A. P. & Walters, N. J. (2000). Basic medical laboratory techniques, 4th edn, Thomson Learning, USA.
32. Varshney, R., Kale, R. K. (1990). Effects of calmodulin antagonists on radiation induced lipid peroxidation in microsomes. Int. J. Rad. Biol., 58:733743.
33. Estridge, B. H., Reynolds, A. P. & Walters, N. J. (2000). Basic medical laboratory techniques, 4th edn, Thomson Learning, USA.
34. Varshney, R. & Kale, R. K. (1990). Effects of calmodulin antagonists on radiation induced lipid peroxidation in microsomes. Int. J. Rad. Biol., 58:733743.
35. Fridorich I. (1989). Superoxide dismutases: An adaptation to a paramagnetic gas. J. Biol. Chem. 1989; 264:7761-7764.
36. Jindal, H., Singh, A., Goyal, R., et al. (2020). Observational study of serum alanine aminotransferase (ALT), serum aspartate aminotransferase (AST) and serum alkaline phosphatase (ALP) levels in type 2 diabetic patients. J Evid Based Med Health, 7(48), 2852-2855.
37. Munkong, N., Ruxsanawet, K., Ariyabukalakorn, V., Mueangchang, W., Sangkham, S., Silangirn, P., Thim-uam, A., Naowaboot, J., Somparn, N. & Yoysungnoen, B. (2024). Hepatoprotective effects of Elaeagnus latifolia fruit extract against acetaminophen-induced hepatotoxicity in mice: Mechanistic insights. Journal of Functional Foods, 114, 2024, 106077.
38. Huang Z., Lin H. W., Zhang Q. & Zong X. (2022). Targeting Alzheimer's disease: the critical crosstalk between the liver and brain. Nutrients 14:4298.
39. Adedara, I. A., Teberen, R., Ebokaiwe, A. P., Ehwerhemuepha, T. & Farombi, E. O. (2012). Induction of oxidative stress in liver and kidney of rats exposed to Nigerian bonny light crude oil. Environ Toxicol. 27:372–379.
40. Xu, S., Li, N., Ning, M. M., Zhou, C. H., Yang, Q. R., & Wang, M. W. (2006). Bioactive compounds from peperomia p ellucida. Journal of natural products, 69(2), 247-250.
41. Gangar, S. C., Sandhir, R., & Koul, A. (2010). Effects of Azadirachta indica on certain hematological parameters during benzo (a) pyrene induced murine forestomach tumorigenesis. Eur Rev Med Pharmacol Sci, 14(12), 1055-1072.
42. De Jong, W. H., Kroese, E. D., Vos, J. G. & Van Loveren, H. (1999). Detection of immunotoxicity of benzo[a]pyrene in a subacute toxicity study after oral exposure in rats. Toxicol Sci., 50 (2):214-20.
43. Arkew, M., Yemane, T., Mengistu, Y., Gemechu, K. & Tesfaye, G. (2021). Hematological parameters of type 2 diabetic adult patients at Debre Berhan Referral Hospital, Northeast Ethiopia: A comparative cross-sectional study. PLoS One. 16(6):e0253286.
44. Habig, W. H., Pabst, M. J., Jakoby, W. B. (1974). Glutathione S-transferase. The first enzymatic step in mercapturic acid formation. J Biol Chem. 1974;249: 7130–7139.
45. Sanocka, D. & Kurpisz, M. (2004). Reactive oxygen species and sperm cells. Reproductive biology and endocrinology, 2(1), 1-7.

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46. Ma, J. Q., Liu, C. M., Qin, Z. H., Jiang, J. H., & Sun, Y. Z. (2011). Ganoderma applanatum terpenes protect mouse liver against benzo (α) pyren-induced oxidative stress and inflammation. Environmental toxicology and pharmacology, 31(3), 460-468.
47. Qamar, W., Khan, A. Q., Khan, R., et al. (2012). Benzo(a)pyrene-induced pulmonary inflammation, edema, surfactant dysfunction, and injuries in rats: Alleviation by farnesol. Exp Lung Res. 2012; 38:19–27.