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Effects of *Spondias mombin* aqueous leaf extract against heavy metals-triggered changes in hematological, antioxidant and semen quality indices in Male Wistar rats

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Abstract

The ameliorative effect of aqueous leaf extract of *Spondias mombin* (SM) on hematological, antioxidant and semen quality indices in Wistar rats exposed to heavy metal toxicants (mercury and cadmium) was investigated. Thirty (30) male rats weighing 120 - 150g randomly divided into ten (10) groups were used in the study. Group-A (Control) received normal rat feeds and distilled water *ad libitum*; Groups-B and -E received $CdCl_2$ and $HgCl_2$ respectively. Groups -C and -F rats were co-administered with $CdCl_2$ or $HgCl_2$ and 500 mg/kg b. w. of SM daily, Groups -D and -G received toxicants and 100 mg/kg b. w. of silymarin, Group-H rats were exposed to a combination of $CdCl_2$ and $HgCl_2$ toxicants. Group-I rats were administered with both toxicants and SM while Group-J received $CdCl_2$, $HgCl_2$ and silymarin. The experiment lasted for 28 days at the end of which semen quality indices, hematological and antioxidant status were assessed using standard biochemical procedures. Results obtained showed significant ($p<0.05$) decreases in serum levels of antioxidant enzymes and sperm quality indices in the $CdCl_2$, $HgCl_2$ and $CdCl_2$ + $HgCl_2$-groups at day 28. However, administration of SM leaf extract led to significant improvement in the levels of glutathione, superoxide dismutase and catalase enzymes in the extract-treated rats. Treatment with SM also improved sperm quality indices, recording as much as 93.3% active sperm cells in group-F. These findings suggest that aqueous leaf extract of *S. mombin* leaves exhibit restorative potentials against heavy metals-triggered changes in hematological, antioxidant and semen quality indices in Wistar rats.

Keywords: *Spondias Mombin*; Leaf Extract; Heavy Metals; Hematology; Antioxidant; Semen Quality

1. Introduction

Globally, a great number of people suffer from metal toxicity through water, air and food contamination. Following accumulation, transportation and compartmentalization into body tissues/cells, metals bind to proteins and nucleic acids, thereby damaging macromolecules and disrupting cellular functions [1]. Heavy metals frequently react with biological systems by losing one or more electrons and forming metal cations that have affinity for nucleophilic sites of vital macromolecules [2]. Studies have reported sex differences in the toxicity of heavy metals [3, 4]. The toxic mechanism of heavy metals function via reactive oxygen species (ROS) generation, enzyme inactivation and suppression of the antioxidant defense. Some heavy metals cause toxicities in a particular pattern, including binding selectively to specific macromolecules [2, 5]. Cadmium (Cd) is known to enter the food chain and undergo bioaccumulation, endangering human health [6]. After absorption, Cd is circulated in blood, bound mainly to blood cells and albumin. It is primarily distributed to the liver and then redistributes progressively to the kidney as cadmium-metallothionein (Cd-MT). After distribution, approximately 50% of the total-body burden is found in the liver and kidney [7]. Nervous, digestive, and renal systems are most commonly affected by Mercury (Hg) exposure; children and pregnant women are most vulnerable to Hg exposure [4]. Mercury represents the third most toxic element on the planet, according to the US Government Agency for Toxic Substances and Diseases Registry [8].

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Spondias mombin (SM) is a deciduous erect tree, commonly found in the tropical Americas, including the West Indies, and has also been naturalized in parts of Africa, including Ghana and some parts of Asia [9]. In ethnomedicine, the stem bark, leaves, and roots of SM have been used for the treatment of various conditions. *S. mombin* possesses antimicrobial and antiviral activities [10–12]. In a previous study, phytochemical screening conducted by Awogbindin et al. [13] indicated that *S. mombin* leaf contain tannins, saponins, alkaloids, flavonoids, phenols, ascorbic acid, niacin, riboflavin and thiamine. Similarly, Njoku and Akumefula [14] quantified the phytochemicals found in leaf extract of *S. mombin* as tannins (3.82%), saponins (7.60%), flavonoids (3.00%), alkaloids (6.00%) and phenols (1%). SM leaves show anti-inflammatory, anthelmintic, hematinic and sedative activities, while its stem bark possesses anti-mycobacterial activity [14-16]. Traditionally, various parts of SM are used for different medicinal purposes, including the treatment of diseases and as forage for domestic animals [17]. In South-Eastern Nigeria, juice extracts from SM leaves are used to facilitate delivery in small ruminants with dystocia arising from uterine inertia, and it has been used in livestock for increased productivity [18].

The present study investigated the ameliorative effects of aqueous leaf extract of *Spondias mombin* on hematological, antioxidant and semen quality indices in Wistar rats exposed to the individual and synergistic effects of mercury and cadmium toxicants.

2. Materials and methods

2.1. Experimental Animals

Thirty (30) adult-male Wistar rats weighing 120-150g obtained from the Animal House of Department of Biochemistry, University of Port Harcourt Nigeria were used in the study. The rats were allowed to acclimatize for 10 days under standard conditions (25 ± 2 °C, 12 h of light and 12h of darkness) and then randomly assigned into ten (10) groups of three (3) rats each. All treatments were carried out via oral gavage for a period of twenty-eight (28) days. Experimental animals were fed standard chow diet and were given access to water ad libitum.

2.2. Chemicals/Reagents/Drug

All reagents used in this study were of analytical grade: (Cadmium chloride ($CdCl_2$), Mercuric chloride ($HgCl_2$), Silymarin, Chloroform, Distilled water and Assay Kits). Silymarin drug was purchased in Port Harcourt Nigeria.

2.3. Plant collection and validation

Spondias mombin (SM) plant was obtained from Omoku Community in Rivers State, Nigeria. The plant was authenticated at the Department of Plant Science and Biotechnology, University of Port Harcourt, Nigeria and assigned voucher number UPH/N/324. Fresh leaves of the plant were used for this study.

2.4. Preparation of aqueous Leaf Extract of Spondias mombin

The fresh leaves of SM obtained were thoroughly washed with distilled water, air-dried 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. [19]. Thirty (30) grams of dry powder was soaked in 300 ml distilled water for three days. The resultant suspension was filtered into sterile beakers, the filtrate was re-filtered using Whatman No.1 filter paper into sterile sample bottles and stored in the refrigerator before use in the study.

2.5. Experimental Grouping

Toxicants, Silymarin drug and *S. mombin* aqueous leaf extract was administered to the experimental rats at varying dosages as follows:

Table 1 Experimental Groups

Experimental Group	Group Description
Group-A Normal Control	Received Normal Rat Feed + Water Only (for 28 days).
Group-B ($CdCl_2$ only)	Received standard feed and water + 1.5 mg/kg b. w. of $CdCl_2$ (for 28 days).
Group-C ($CdCl_2$ + SM)	Received standard feed and water + 1.5 mg/kg b. w. of $CdCl_2$ + 500mg/kg b. w. of SM daily (for 28 days).
Group-D ($CdCl_2$ + Silymarin)	Received standard feed and water + 1.5 mg/kg b. w. of $CdCl_2$ + 100 mg/kg b. w. of silymarin daily (for 28 days).
Group-E ($HgCl_2$ only)	Received standard feed and water + 1.2mg/kg b. w. of $HgCl_2$ (for 28 days).
Group-F ($HgCl_2$ + SM)	Received standard feed and water + 1.2mg/kg b. w. of $HgCl_2$ + 500mg/kg b. w. of SM daily (for 28 days).
Group-G ($HgCl_2$ + Silymarin)	Received standard feed and water + 1.2mg/kg b. w. of $HgCl_2$ + 100 mg/kg b. w. of silymarin daily (for 28 days).
Group-H ($CdCl_2$ + $HgCl_2$)	Received standard feed and water + 1.5 mg/kg b. w. of $CdCl_2$ + 1.2mg/kg b. w. of $HgCl_2$ (for 28 days).
Group-I ($CdCl_2$ + $HgCl_2$ + SM)	Received standard feed and water + 1.5 mg/kg b. w. of $CdCl_2$ + 1.2mg/kg b. w. of $HgCl_2$ + 500mg/kg b. w. of SM daily (for 28 days).
Group-J ($CdCl_2$ + $HgCl_2$ + Silymarin)	Received standard feed and water + 1.5 mg/kg b. w. of $CdCl_2$ + 1.2mg/kg b. w. of $HgCl_2$ + 100 mg/kg b. w. of silymarin daily (for 28 days).

2.6. Acute toxicity study for *Spondias mombin* leaf extract

Acute toxicity level for *S. mombin* leaf extract was considered following previous findings by Nwidu et al. [20].

2.7. Biochemical Assays

2.7.1. Determination of Hematological Parameters

The following parameters were analyzed: packed cell volume (PCV), hemoglobin (Hb), platelets, red blood cell count (RBC) and white blood cell count (WBC) - neutrophil, lymphocytes, monocytes, eosinophil and basophil. Blood samples used for analysis of hematological parameters were collected in EDTA bottles and analyzed using the Mindray Auto Hematology Analyzer (BC-5200, USA).

2.7.2. Antioxidant Assays

Catalase activity was assayed using the method of Aebi [21]. Superoxide dismutase (SOD) activity was assayed using the method described by Fridorich [22] while the concentration of glutathione was determined according to the method of Habig et al. [23].

2.7.3. Semen collection and Analysis

Semen collection was done by the electro-ejaculation method described by Oyeyemi et al. [24]. Sperm quality indices were evaluated using a method described by Ajani et al. [25].

2.8. Histological Investigation

Specimens of the testis were cut into pieces and fixed in 10% formalin, routinely processed for dehydration, and embedded in paraffin wax. Sections (5 mm-thick) were cut, fixed onto glass slides, and stained with hematoxylin and eosin for light microscopic examination [26]. The slides were examined under a high-resolution microscope (Canada balsam) at a magnification of x400.

2.9. 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.

3. Results and discussion

3.1. Effects of *S. mombin* aqueous leaf extract on hematological parameters

Tables 2 (a) and (b) show the effects of aqueous extract of *S. mombin* leaves on hematological parameters during heavy metal-triggered toxicity in Wistar rats. Administration of the leaf extract led to a significant increase in the number of white blood cells (WBC) in Group-F ($HgCl_2$ + SM) as compared to Group-B ($CdCl_2$ only) and Group-E ($HgCl_2$ only). There was significant ($p<0.05$) reduction in PCV, Hemoglobin and RBC in the $CdCl_2$ only group as compared to other groups in the study.

3.2. Effects of *S. mombin* aqueous leaf extract on antioxidant enzymes

Table 3 shows the effects of aqueous extract of *S. mombin* leaf on antioxidant enzymes in heavy metal-triggered toxicity in Wistar rats**.** There were significant decreases in the activities of antioxidant enzymes SOD, CAT and GSH in Group-B ($CdCl_2$ only) and Group-E ($HgCl_2$ only) at day 28. There was significant ($p<0.05$) improvement in the activities of the antioxidant level indicators in Group-C ($CdCl_2$ + SM), Group-F ($HgCl_2$ + SM) and Group-I ($CdCl_2$ + $HgCl_2$ + SM)**,** following treatment with *S. mombin* leaf extract. Also, significant increases in the activities of SOD, CAT and GSH were also observed in Group-D ($CdCl_2$ + Silymarin), Group-G ($HgCl_2$ + Silymarin) and Group-J ($CdCl_2$ + $HgCl_2$ + Silymarin) as a result of treatment with the drug, Silymarin.

3.3. Effects of *S. mombin* aqueous leaf extract on sperm quality parameters

Tables 4 (a) & (b) show the effects of ethanol extract of *S. mombin* leaf on sperm quality parameters in heavy metal-induced toxicity in Wistar rats. The leaf extract improved % active sperm cells as observed Group-C ($CdCl_2$ + SM) and Group-F ($HgCl_2$ + SM) at day 28. Groups -C and -F also showed significant ($p<0.05$) increase in % motility in comparison with Group-B ($CdCl_2$ only) and Group-E ($HgCl_2$ only) groups.

3.4. Results for Histological Studies

3.4.1. Testis Photomicrographs

Plates T-G1 to T-G10 are light microscope photographs of liver paraffin sections obtained from groups 1 -10 at day 28, stained with hematoxylin and eosin.

Table 2 (a) Effects of Cadmium and Mercury-Induced Toxicities on Hematological Indices

Parameters	**PVC (%)**	**HB (g/dl)**	**WBC ($x10^3$ /mm^3)**	**PLT (10^9/L)**	**RBC (10^9/L)**
Group-A Normal Control	46.00 ± 1.53	15.33 ± 0.49	8.33 ± 0.33	524.00 ± 122.20	6.00 ± 0.49
Group-B ($CdCl_2$ only)	37.67 ± 5.90^{a}	12.57 ± 1.96^{a}	8.93 ± 2.59^{d}	290.67 ± 78.16^{a}	5.10 ± 0.72^{d}
Group-C ($CdCl_2$ + SM)	44.67 ± 1.33^{d}	14.87 ± 0.433^{c}	8.83 ± 0.82^{d}	136.33 ± 26.49^{bd}	5.37 ± 0.23^{c}
Group-D ($CdCl_2$ + Silymarin)	40.00 ± 2.89^{b}	14.90 ± 0.90^{c}	5.67 ± 0.63^{ce}	230.33 ± 69.57^{c}	5.20 ± 0.12^{d}
Group-E ($HgCl_2$ only)	45.00 ± 1.16^{d}	15.00 ± 0.40^{d}	7.67 ± 2.94^{d}	236.00 ± 62.43^{a}	5.27 ± 0.23^{c}
Group-F ($HgCl_2$ + SM)	43.00 ± 0.58^{c}	14.33 ± 0.20^{c}	10.17 ± 1.36^{bd}	278.67 ± 76.41^{b}	5.13 ± 0.33^{b}
Group-G ($HgCl_2$ + Silymarin)	46.67 ± 4.41^{e}	16.53 ± 0.96^{e}	4.77 ± 1.75^{ce}	221.00 ± 10.02^{c}	7.10 ± 0.61^{e}
Group-H ($CdCl_2$ + $HgCl_2$)	45.33 ± 0.33^{d}	15.10 ± 0.10^{d}	8.57 ± 2.11^{d}	153.3 ± 13.42^{a}	6.23 ± 0.34^{de}
Group-I ($CdCl_2$ + $HgCl_2$ + SM)	42.33 ± 3.71^{b}	14.13 ± 1.23^{c}	5.10 ± 1.23^{bd}	180.00 ± 20.79^{b}	5.67 ± 0.92^{d}
Group-J ($CdCl_2$ + $HgCl_2$ + Silymarin)	40.00 ± 5.77^{ce}	14.03 ± 2.05^{c}	5.97 ± 1.38^{ce}	224.67 ± 44.89^{ce}	6.27 ± 1.59^{e}

Values are reported as Mean ± Standard Deviation, (n =3). Treatment with same or similar superscripts "a,b,c,d,e" are not statistical significantly difference (P≤ 0.05) from one another while treatments with different superscript are statistically significantly different from one another.
*Pack Cell Volume – PCV; Hemoglobin – Hb; White Blood Cell – WBC; Platelet -PLT; Red Blood Cell – RBC

Table 2 (b) Effects of Cadmium and Mercury-Induced Toxicities on WBC Indices

Parameters	NEU (%)	LYM (%)	MON (%)	EOS (%)	BAS (%)
Group-A Normal Control	29.00 ± 2.08	63.00 ± 2.08	5.67 ± 1.20	2.33 ± 0.88	0.00 ± 0.00
Group-B ($CdCl_2$ only)	25.00 ± 2.89^{a}	67.00 ± 4.16^{d}	6.00 ± 2.31^{d}	1.00 ± 1.00^{a}	0.00 ± 0.00
Group-C ($CdCl_2$ + SM)	21.33 ± 3.76^{b}	71.00 ± 3.0^{b}	6.00 ± 1.73^{d}	1.67 ± 0.33^{b}	0.00 ± 0.00
Group-D ($CdCl_2$ + Silymarin)	15.67 ± 1.45^{c}	77.67 ± 2.03^{ce}	3.33 ± 0.88^{ce}	2.33 ± 0.33^{e}	0.00 ± 0.00
Group-E ($HgCl_2$ only)	27.67 ± 5.78^{d}	63.67 ± 6.74^{d}	5.33 ± 1.45^{c}	1.33 ± 1.33^{a}	0.00 ± 0.00
Group-F ($HgCl_2$ + SM)	29.00 ± 1.16^{e}	62.67 ± 1.20^{cd}	5.33 ± 0.88^{c}	3.00 ± 1.73^{bd}	0.00 ± 0.00
Group-G ($HgCl_2$ + Silymarin)	20.67 ± 1.76^{c}	73.00 ± 1.73^{ce}	5.00 ± 0.58^{b}	3.00 ± 1.73^{ce}	0.00 ± 0.00
Group-H ($CdCl_2$ + $HgCl_2$)	18.33 ± 2.73^{a}	77.67 ± 2.19^{a}	3.33 ± 0.88^{a}	0.67 ± 0.37^{a}	0.00 ± 0.00
Group-I ($CdCl_2$ + $HgCl_2$ + SM)	18.33 ± 2.73^{b}	66.00 ± 3.01^{d}	2.33 ± 1.33^{b}	1.67 ± 0.67^{bd}	0.00 ± 0.00
Group-J ($CdCl_2$ + $HgCl_2$ + Silymarin)	24.33 ± 2.60^{c}	63.33 ± 2.85^{e}	8.00 ± 1.00^{ce}	1.67 ± 1.20^{ce}	0.00 ± 0.00

Values are reported as Mean ± Standard Deviation, (n =3). Treatment with same or similar superscripts "a,b,c,d,e" are not statistical significantly difference (P≤ 0.05) from one another while treatments with different superscript are statistically significantly different from one another.
*Neutrophil – NEU; Lymphocytes – LYM; Monocytes – MON; Eosinophil – EOS; Basophil – BAS

Table 3 Effects of Cadmium and Mercury-Induced Toxicities on Oxidative Stress Markers

Parameters	SOD (IU/I)	CATALASE (Kat f)	GLUTHATHIONE (µg/g)
Group-A Normal Control	154.33 ± 10.23	4.58 ± 0.27	34.67 ± 4.10
Group-B ($CdCl_2$ only)	114.00 ± 6.25^{a}	2.60 ± 0.15^{a}	25.0 ± 7.23^{a}
Group-C ($CdCl_2$ + SM)	135.67 ± 4.41^{bd}	3.50 ± 0.21^{bd}	45.0 ± 21.6^{bd}
Group-D ($CdCl_2$ + Silymarin)	233.33 ± 59.38^{ce}	3.30 ± 0.42^{ce}	162.67 ± 15.92^{ce}
Group-E ($HgCl_2$ only)	149.67 ± 31.52^{a}	2.33 ± 0.12^{a}	36.67 ± 3.71
Group-F ($HgCl_2$ + SM)	197.00 ± 13.65^{bd}	3.53 ± 0.20^{bd}	58.33 ± 10.93^{bd}
Group-G ($HgCl_2$ + Silymarin)	207.33 ± 13.64^{ce}	3.70 ± 0.15^{ce}	174.33 ± 4.49^{ce}
Group-H ($CdCl_2$ + $HgCl_2$)	185.00 ± 24.09^{a}	3.07 ± 0.09^{a}	38.33 ± 8.09
Group-I ($CdCl_2$ + $HgCl_2$ + SM)	193.33 ± 16.68^{bd}	3.77 ± 0.09^{b}	64.33 ± 2.40^{bd}
Group-J ($CdCl_2$ + $HgCl_2$ + Silymarin)	200.00 ± 11.846^{ce}	3.53 ± 0.32^{c}	86.00 ± 22.37^{ce}

Values are reported as Mean ± Standard Deviation, (n =3). Treatment with same or similar superscripts "a,b,c,d,e" are not statistical significantly difference (P≤ 0.05) from one another while treatments with different superscript are statistically significantly different from one another.

Table 4 (a) Effects of Cadmium and Mercury-Induced Toxicities on Semen Characteristics

Parameters	Motility Cells (%)	Active Cells (%)	Sluggish Cells (%)	Dead Cells (%)	Head Defects (%)
Group-A Normal Control	94.00 ± 4.51	86.67 ± 6.01	7.33 ± 1.76	4.33 ± 2.85	0.67 ± 0.67
Group-B ($CdCl_2$ only)	36.67 ± 12.02^{a}	25.00 ± 13.23^{a}	11.67 ± 1.67^{a}	60.00 ± 10.0^{a}	0.00 ± 0.00
Group-C ($CdCl_2$ + SM)	75.00 ± 13.23^{b}	68.33 ± 12.02^{bd}	6.67 ± 1.67^{d}	31.67 ± 10.14^{b}	1.67 ± 1.67^{c}
Group-D ($CdCl_2$ + Silymarin)	46.67 ± 19.22^{c}	36.67 ± 19.22^{ce}	10.00 ± 0.00^{c}	35.00 ± 17.56^{c}	0.33 ± 0.33^{b}
Group-E ($HgCl_2$ only)	88.00 ± 7.00^{d}	80.00 ± 7.64^{d}	8.00 ± 1.53^{d}	12.00 ± 7.00^{a}	0.00 ± 0.00
Group-F ($HgCl_2$ + SM)	98.00 ± 1.53^{d}	93.33 ± 3.33^{d}	4.67 ± 2.60^{b}	2.00 ± 1.53^{b}	0.67 ± 0.67^{a}
Group-G ($HgCl_2$ + Silymarin)	18.33 ± 15.90^{c}	15.00 ± 0.41^{ce}	6.67 ± 4.41^{d}	73.33 ± 14.53^{c}	0.33 ± 0.33^{b}
Group-H ($CdCl_2$ + $HgCl_2$)	81.67 ± 4.41^{a}	75.00 ± 5.77^{a}	6.67 ± 1.67^{a}	18.33 ± 4.41^{a}	0.00 ± 0.00
Group-I ($CdCl_2$ + $HgCl_2$ + SM)	53.33 ± 28.04^{b}	48.33 ± 26.19^{bd}	5.00 ± 2.89^{b}	46.67 ± 28.04^{b}	0.33 ± 0.33^{b}
Group-J ($CdCl_2$ + $HgCl_2$ + Silymarin)	8.33 ± 6.01^{c}	5.00 ± 2.89^{ce}	5.00 ± 2.89^{c}	93.33 ± 6.67^{c}	0.00 ± 0.00

Values are reported as Mean ± Standard Deviation, (n =3). Treatment with same or similar superscripts "a,b,c,d,e" are not statistical significantly difference (P≤ 0.05) from one another while treatments with different superscript are statistically significantly different from one another.

Table 4 (b) Effects of Cadmium and Mercury-Induced Toxicities on Semen Characteristics

Parameters	Tail Defects (%)	Mid Pieces (%)	Viable (%)	Non-Viable (%)	Total Sperm Count (%)
Group-A Normal Control	1.33 ± 0.33	0.67 ± 0.33	97.33 ± 1.20	2.67 ± 1.20	342.00 ± 106.33
Group-B ($CdCl_2$ only)	1.00 ± 0.00^{d}	3.67 ± 3.18^{a}	95.33 ± 3.18^{cd}	4.67 ± 3.18^{a}	323.67 ± 79.82
Group-C ($CdCl_2$ + SM)	0.33 ± 0.33^{b}	1.00 ± 0.58^{d}	98.67 ± 0.88^{d}	1.33 ± 0.88^{bd}	233.67 ± 37.25^{bd}
Group-D ($CdCl_2$ + Silymarin)	1.33 ± 0.33^{a}	1.00 ± 0.58^{e}	97.67 ± 0.33^{de}	1.67 ± 0.33^{ce}	234.33 ± 29.19^{ce}
Group-E ($HgCl_2$ only)	1.33 ± 1.33^{a}	0.33 ± 0.33	98.33 ± 1.67^{e}	1.67 ± 1.67^{a}	186.33 ± 40.09^{a}
Group-F ($HgCl_2$ + SM)	0.67 ± 0.33^{b}	1.33 ± 0.33^{bd}	98.00 ± 0.58^{e}	2.00 ± 0.58^{d}	171.33 ± 28.62^{bd}
Group-G ($HgCl_2$ + Silymarin)	1.33 ± 0.33^{a}	4.33 ± 0.67^{ce}	93.67 ± 0.89^{c}	7.00 ± 0.58^{ce}	81.33 ± 17.98^{ce}
Group-H ($CdCl_2$ + $HgCl_2$)	1.00 ± 0.58^{d}	3.00 ± 3.00^{a}	96.33 ± 3.67^{d}	3.67 ± 3.67^{a}	224.00 ± 45.43^{a}
Group-I ($CdCl_2$ + $HgCl_2$ + SM)	0.67 ± 0.67^{b}	0.00 ± 0.00	99.33 ± 0.67^{e}	0.67 ± 0.67^{bd}	125.33 ± 49.33^{bd}
Group-J ($CdCl_2$ + $HgCl_2$ + Silymarin)	0.67 ± 0.33^{c}	0.67 ± 0.33^{e}	98.00 ± 1.16^{e}	1.33 ± 0.67^{ce}	207.00 ± 77.37^{ce}

Values are reported as Mean ± Standard Deviation, (n =3). Treatment with same or similar superscripts "a,b,c,d,e" are not statistical significantly difference (P≤ 0.05) from one another while treatments with different superscript are statistically significantly different from one another.

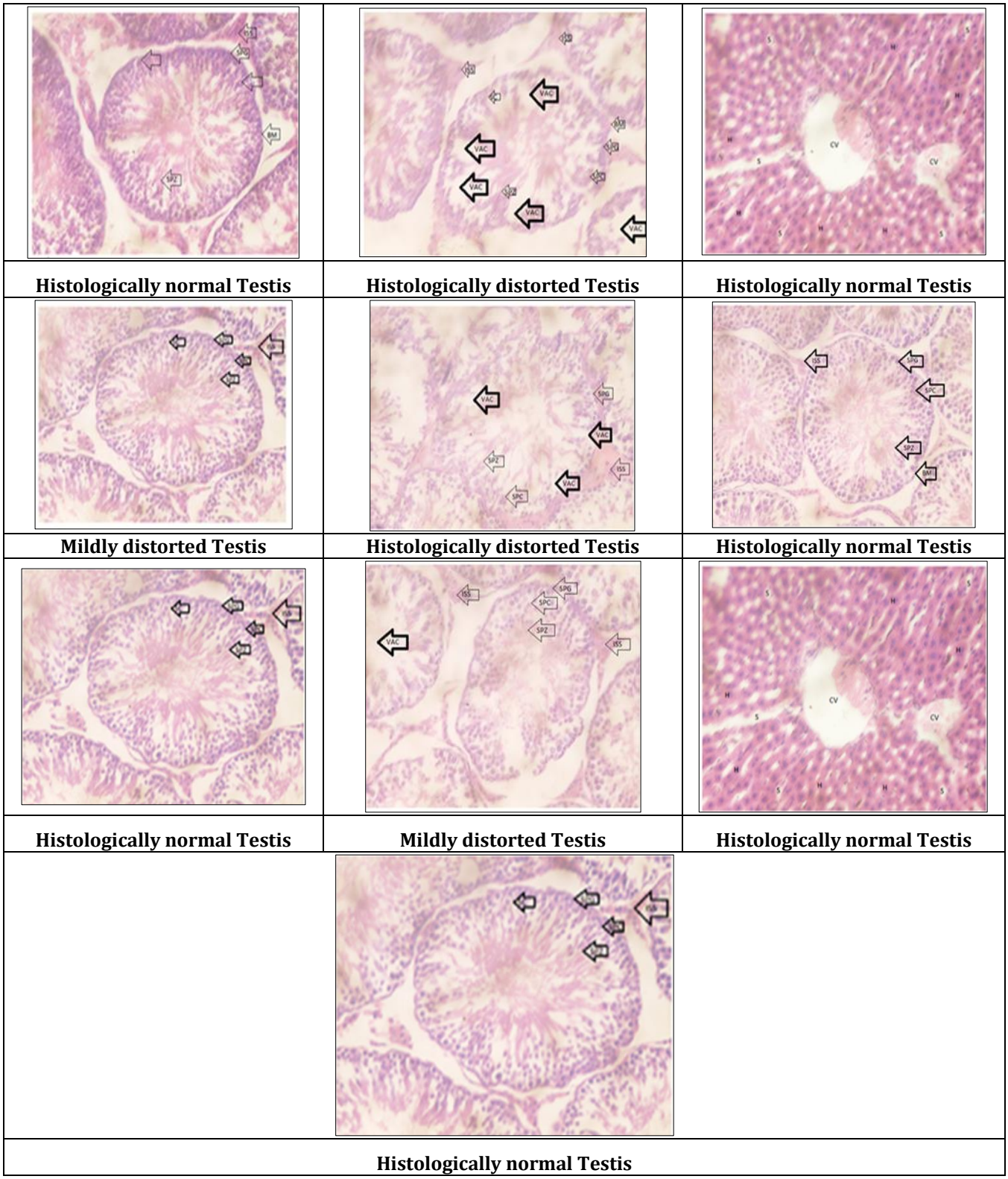

Figure 1 Plates T-G1 to T-G10. Light microscope photographs of liver paraffin sections (H & E stained), (Mag *400)

Cadmium and mercury are recognized to have destructive impacts on most organ systems [27]. The present study investigated the individual and synergistic effects of cadmium and mercury on Wistar rats. Findings showed that exposure to both heavy metals negatively affected hematological, antioxidant and semen quality indices in the experimental rats when compared to control groups.

Assessment of haematological parameters is very useful. Haematological parameters are of diagnostic significance in the routine clinical evaluation of the state of health [29 - 30]. Hemoglobin is known to be a protein used by erythrocytes to distribute oxygen to other tissues and cells in the body [31, 32]. Reduction in erythrocyte number and haemoglobin content is indicative of defective hematopoiesis. Increases in haemoglobin level is normally followed by corresponding increase in haematocrite level [28]. Levels of leukocytes significantly ($p<0.05$) increased in Group-F ($HgCl_2$ + SM), as compared to Group-B ($CdCl_2$ only) and Group-E ($HgCl_2$ only). Leukocytosis may be directly proportional to the severity of the causative stress condition [33, 34]. The aqueous extract of *Spondias mombin* (SM) administered induced desirable alterations in packed cell volume (PCV), haemoglobin concentration (Hb), red blood cell (RBC) and white blood cell (WBC) counts in the treated animals. The improvement in red blood cell (RBC) count as shown in this study, reveal the probable capability of *S. mombin* leaf to improve anaemic conditions in animals. Also, the increase in lymphocyte counts following administration of *S. mombin* leaf extract depicts the anti-inflammatory and anti-stress properties of the fraction [35]. These beneficial effects of the methanol fraction of *S. mombin* on the haematological profile may be attributed to the presence of flavonoids, tannins, vitamin C, iron and other substances with antioxidant and erythropoietic stimulatory properties in the plant [36 – 38]. The leaf extract under study was able to significantly ($p<0.05$) boost the immune system of experimental animals as observed in increase in WBC, Hb, and lymphocytes levels. It can be inferred that *S. mombin* leaf extract showed potentials to cushion detrimental effects of cadmium and mercury. This finding corroborates previous research works by Gangar et al. [39] and De Jong et al [40].

Dysregulation of the oxidative status, via excessive production of oxidants or impairment of antioxidant activity, is often considered one of the consequences of heavy metal toxicity and poisoning in animals and humans [41]. Superoxide dismutase (SOD) accelerates the conversion of endogenous cytotoxic superoxide radicals to H_2O_2 while the metabolic role of catalase (CAT) is to eliminate H_2O_2 thereby protecting the cells. Glutathione (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 [42].

In the present study, heavy metals-exposed rats showed marked decreases in GSH, SOD and CAT levels. However, treatment with *S. mombin leaf* extract was able to restore this effect. The ameliorative and therapeutic effects of *S. mombin* in heavy metals-triggered toxicity may be attributed to the enhancement of antioxidant defense mechanism by the extract [32].

Heavy metals exposure to the experimental animals resulted in depletion of testicular and epididymal functions in animals. This finding corroborates a previous toxicology study by Reddy et al. [43] who assessed reproductivity toxicity in adult male Wistar rat and concluded that chronic exposure to toxicants alter steroidogenesis and spermatogenesis, leading to depleted fertility in adult male rats. Results in the present study reveal that heavy metal exposure caused acute and significant ($p<0.05$) decreases in sperm quality indices. This finding is similar to previous report by Oloye et al. [44] where they noted improvement in sperm parameters following a 14-day treatment with 400mg/kg *S. mombin* aqueous leaf extract. Martin and Touaibia [45] reported in their review that plant extracts rich in flavonoids, such as those found in the leaves of *S. mombin*, can improve sperm parameters. These flavonoids could potentially influence the production of androgen (majorly testosterone) by the Leydig cells [46]. SM aqueous leaf extract was able to ameliorate the detrimental effects of the heavy metals on male fertility indices. This was further confirmed by histological plates which indicated histologically normal testis tissues in the *S. mombin* treated groups.

4. Conclusion

Findings from the present study have shown that *Spondias mombin* leaves exhibit promising haematological, antioxidant potency and fertility restorative potentials due to the wide array of phytochemicals reported to be present in *S. mombin* leaves.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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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