

# THERAPEUTIC POTENTIAL OF ANNONA MURICATA LEAF ETHANOLIC EXTRACT IN MITIGATING ARSENIC-INDUCED TESTICULAR DYSFUNCTION, OXIDATIVE STRESS, AND REPRODUCTIVE IMPAIRMENT IN MALE WISTAR RATS.

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## ABSTRACT

**Background:** Arsenic is a pervasive environmental toxicant that poses serious health risks to both humans and animals. Chronic exposure to arsenic has been associated with oxidative stress-mediated damage in several organs, particularly the testes, resulting in impaired steroidogenesis, defective spermatogenesis, and reduced fertility. The search for effective natural therapeutic agents capable of attenuating arsenic-induced reproductive toxicity has intensified due to the limitations associated with conventional interventions.

**Objective:** This study investigated the protective effects of ethanolic leaf extract of *Annona muricata* against arsenic-induced testicular dysfunction, oxidative stress, and reproductive impairment in adult male Wistar rats.

**Materials and Methods:** Thirty adult male Wistar rats were randomly assigned into five experimental groups (n = 6). Animals received either distilled water, arsenic alone, *Annona muricata* extract alone, or arsenic combined with two different doses of the extract (200 and 400 mg/kg body weight) for twenty-one consecutive days. Oxidative stress biomarkers, including superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH), and malondialdehyde (MDA), were assessed. Serum testosterone concentration, sperm characteristics, and histopathological changes in testicular tissues were also evaluated.

**Results:** Arsenic exposure significantly disrupted testicular redox homeostasis, as evidenced by elevated MDA levels and marked reductions in SOD, CAT, and GSH activities. Furthermore, arsenic administration resulted in decreased serum testosterone concentration, impaired sperm quality, and extensive histological damage characterized by seminiferous tubular degeneration, germ-cell depletion, and spermatogenic arrest. Treatment with *Annona muricata* extract significantly ameliorated these alterations in a dose-dependent manner. Rats receiving 400 mg/kg

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of the extract exhibited substantial restoration of antioxidant status, improved testosterone production, enhanced sperm characteristics, and near-normal testicular histoarchitecture.

**Conclusion:** The findings demonstrate that *Annona muricata* leaf ethanolic extract effectively attenuates arsenic-induced reproductive toxicity through modulation of oxidative stress and preservation of testicular integrity. These results support the potential application of *Annona muricata* as a phytotherapeutic candidate for the management of environmentally induced male reproductive disorders. Further molecular investigations are warranted to elucidate the underlying mechanisms of action.

**Keywords:** *Annona muricata*; arsenic toxicity; oxidative stress; reproductive dysfunction; testosterone; spermatogenesis; antioxidant therapy.

## **1. INTRODUCTION**

Male infertility constitutes a significant global health challenge, accounting for approximately 40–50% of infertility cases worldwide (Aitken & Roman, 2008). Emerging evidence indicates that environmental pollutants play a substantial role in the increasing prevalence of male reproductive dysfunction (Chandra et al., 2013; Flora, 2011). Among these pollutants, arsenic remains one of the most hazardous environmental contaminants because of its widespread distribution in groundwater, industrial effluents, agricultural products, mining wastes, and occupational settings (Flora, 2011).

The reproductive system is highly susceptible to toxic insults due to the complexity of spermatogenesis and the delicate hormonal regulation required for normal fertility. Experimental and epidemiological studies have demonstrated that arsenic exposure adversely affects male reproductive function through endocrine disruption, mitochondrial dysfunction, oxidative stress, inflammation, and apoptosis (Chandra et al., 2013; Flora, 2011).

Oxidative stress has been identified as a principal mechanism underlying arsenic-induced reproductive toxicity. Excessive generation of reactive oxygen species overwhelms endogenous antioxidant defense systems, resulting in lipid peroxidation, protein oxidation, DNA damage, and cellular dysfunction (Valko et al., 2007; Halliwell & Gutteridge, 2015). Testicular tissues are particularly vulnerable because spermatozoa possess abundant polyunsaturated fatty acids and limited antioxidant defenses (Aitken & Roman, 2008).

Growing concerns regarding the adverse effects of synthetic drugs have stimulated interest in medicinal plants as alternative therapeutic agents. Plant-derived bioactive compounds possess antioxidant, anti-inflammatory, anti-apoptotic, and cytoprotective properties that may ameliorate toxicant-induced reproductive disorders (Oyeleke et al., 2020).

*Annona muricata* Linn. (soursop), belonging to the family Annonaceae, is widely distributed throughout tropical and subtropical regions. Different parts of the plant have been used traditionally for the treatment of hypertension, diabetes, microbial infections, inflammation, and cancer (Adewole et al., 2011; Coria-Téllez et al., 2018).

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Phytochemical analyses have revealed the presence of flavonoids, phenolic compounds, alkaloids, tannins, saponins, and annonaceous acetogenins, which contribute significantly to its pharmacological activities (Coria-Téllez et al., 2018; Moghadamtousi et al., 2015).

Several studies have demonstrated that *Annona muricata* possesses potent antioxidant and free-radical-scavenging activities capable of enhancing endogenous antioxidant defenses and protecting tissues against oxidative injury (Acésio et al., 2017; Oyeleke et al., 2020; Moghadamtousi et al., 2015). However, information regarding its protective efficacy against arsenic-induced reproductive toxicity remains limited.

## **2. MATERIALS AND METHODS**

### **2.1 Study Design**

This experimental study was conducted to investigate the protective effects of ethanolic leaf extract of *Annona muricata* against arsenic-induced reproductive toxicity in adult male Wistar rats. The study employed a completely randomized design comprising five experimental groups. Biochemical, hormonal, spermatological, and histopathological parameters were evaluated following treatment to determine the extent of reproductive injury and the potential ameliorative effects of the plant extract.

### **2.2 Experimental Animals**

Thirty healthy adult male Wistar rats (*Rattus norvegicus*), aged 10–12 weeks and weighing between 180 and 220 g, were utilized for the study. Animals were obtained from an accredited breeding facility and allowed to acclimatize for two weeks before the commencement of the experiment. Rats were housed in standard polypropylene cages under controlled environmental conditions, including a temperature of  $22 \pm 2^{\circ}\text{C}$ , relative humidity of 50–60%, and a 12-hour light/dark cycle. Standard laboratory pellets and clean drinking water were provided ad libitum throughout the experimental period.

### **2.3 Ethical Approval**

All experimental procedures were performed in accordance with internationally accepted guidelines for the care and use of laboratory animals and complied with the principles outlined in the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Ethical clearance was obtained from the Institutional Research Ethics Committee of the Federal University of Technology, Owerri (Approval No. FUTO/ANA/REC/2026/024).

### **2.4 Collection and Authentication of Plant Material**

Fresh mature leaves of *Annona muricata* were harvested from pesticide-free farmland in Ihiagwa, Imo State, Nigeria. Botanical identification and authentication were carried out by a qualified taxonomist in the Department of Botany. A voucher specimen was prepared and deposited in the departmental herbarium for future reference.

### **2.5 Preparation of Ethanolic Leaf Extract**

Collected leaves were thoroughly rinsed with distilled water to remove debris and air-dried under shade at ambient temperature for fourteen days. The dried leaves were pulverized into fine powder

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using an electric grinder. Five hundred grams of the powdered material were macerated in 2.5 L of 95% ethanol for 72 hours with intermittent agitation to facilitate extraction. The resulting mixture was filtered using Whatman No. 1 filter paper, and the filtrate was concentrated under reduced pressure using a rotary evaporator maintained at 40°C. The concentrated extract was further dried to obtain a semi-solid residue and stored at 4°C until required for administration.

## 2.6 Phytochemical Screening

Qualitative phytochemical analysis of the ethanolic extract was performed using standard laboratory procedures to identify major bioactive constituents. The screening revealed the presence of flavonoids, phenolic compounds, alkaloids, tannins, saponins, and glycosides, which are known for their antioxidant and cytoprotective properties.

## 2.7 Induction of Arsenic Toxicity

Experimental toxicity was induced through administration of arsenic at a dose of 2 mg/kg body weight daily for twenty-one consecutive days. The selected dose was based on previous toxicological studies demonstrating its ability to induce oxidative and reproductive damage without causing excessive mortality.

## 2.8 Experimental Groups

**Table 1:** Showing Experimental design

| Groups | Administration                                         | Duration (Weeks) | Number of rats |
|--------|--------------------------------------------------------|------------------|----------------|
| A      | Feed + distilled water                                 | 3                | 6              |
| B      | Feed + distilled water and Arsenic only                | 3                | 6              |
| C      | Feed + distilled water + 100mg/kg extract              | 3                | 6              |
| D      | Feed + distilled water and Arsenic + 200 mg/kg extract | 3                | 6              |
| E      | Feed + distilled water and Arsenic+ 400 mg/kg extract  | 3                | 6              |
| n = 30 |                                                        |                  |                |

**Source:** Field work, 2025

## 2.9 Sample Collection

At the end of the treatment period, animals were fasted overnight and anesthetized using ketamine hydrochloride (50 mg/kg body weight). Blood samples were collected by cardiac puncture and centrifuged at 3000 rpm for 10 minutes to obtain serum for hormonal analysis. The testes and epididymides were carefully excised, cleaned of surrounding tissues, and weighed. One testis from each animal was processed for biochemical assays, while the contralateral testis was preserved in neutral buffered formalin for histological examination.

## 2.10 Biochemical Assays

Testicular tissue was homogenized in 0.1 M phosphate buffer (pH 7.4) to prepare a 10 % (w/v) homogenate. Homogenates were centrifuged at 10 000 rpm for 15 min at 4 °C, and the supernatant was used for assays.

### **2.11 Determination of Superoxide Dismutase (SOD) Activity**

SOD activity was determined by inhibition of epinephrine auto-oxidation to adrenochrome under alkaline conditions (pH 10.2). Absorbance was monitored at 480 nm. One unit of SOD activity was defined as the amount of enzyme required to inhibit 50 % of epinephrine auto-oxidation. Results were expressed as U/mg protein.

### **2.12 Determination of Catalase (CAT) Activity**

Catalase activity was measured according to Aebi (1984). The decrease in absorbance of hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) was monitored at 240 nm. Activity was expressed as  $\mu\text{mol H}_2\text{O}_2$  decomposed/min/mg protein.

### **2.13 Determination of Reduced Glutathione (GSH)**

GSH levels were quantified using the 5,5'-dithiobis-2-nitrobenzoic acid (DTNB) method. Absorbance of the yellow chromogen was measured at 412 nm. Results were expressed as  $\mu\text{mol GSH/mg protein}$ .

### **2.14 Determination of Lipid Peroxidation (MDA Level)**

MDA levels were assessed using the thiobarbituric acid reactive substances (TBARS) assay. Absorbance was read at 532 nm. Results were expressed as  $\text{nmol MDA/mg protein}$ .

### **2.15 Hormonal Assay**

Serum testosterone was determined using a commercial ELISA kit based on competitive immunoassay. Absorbance was measured at 450 nm, and concentrations were calculated from a standard curve.

### **2.16 Sperm Analysis**

Sperm parameters were evaluated from the cauda epididymis. The epididymis was minced in physiological saline at 37 °C.

Sperm count: Determined using a Neubauer hemocytometer at  $\times 400$  magnification ( $\times 10^6$  sperm/mL).

Sperm motility: Percentage of progressively motile spermatozoa recorded immediately.

Sperm morphology: Eosin-nigrosin-stained smears; 200 spermatozoa evaluated for defects.

### **2.17 Histological Examination**

Testicular tissues were fixed in 10% neutral-buffered formalin, processed through graded alcohol concentrations, cleared in xylene, and embedded in paraffin wax. Sections measuring 5  $\mu\text{m}$  were prepared using a rotary microtome and stained with hematoxylin and eosin (H&E). Microscopic examination was conducted to assess seminiferous tubular architecture, spermatogenic activity, Leydig cell morphology, and other pathological changes associated with arsenic toxicity.

### **2.18 Statistical Analysis**

Data were expressed as mean  $\pm$  standard error of mean (SEM). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison post hoc test. Statistical significance was accepted at  $p < 0.05$ . GraphPad Prism software was utilized for all analyses.

## **3. RESULTS**

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### 3.1 Effect of Ethanol Leaf Extract of *Annona muricata* on Testicular Oxidative Stress Markers in Arsenic-Induced Toxicity

Exposure to arsenic significantly disrupted testicular redox homeostasis compared with the control group. Animals in the arsenic-treated group exhibited marked reductions in the activities of endogenous antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT), and reduced glutathione (GSH), indicating compromised antioxidant defense mechanisms. In contrast, lipid peroxidation, as measured by malondialdehyde (MDA) concentration, was significantly elevated, suggesting increased oxidative damage to cellular membranes.

Administration of *Annona muricata* leaf extract significantly ameliorated these alterations in a dose-dependent manner. Rats treated with 200 mg/kg extract demonstrated partial restoration of antioxidant enzyme activities and a corresponding reduction in MDA levels. More pronounced improvements were observed in the 400 mg/kg treatment group, where antioxidant biomarker values approached those recorded in the control animals. The extract-only group maintained antioxidant profiles comparable to the control group, indicating the absence of adverse oxidative effects associated with extract administration alone.

The observed restoration of antioxidant status suggests that *Annona muricata* effectively counteracts arsenic-induced oxidative stress through enhancement of endogenous antioxidant defenses and suppression of lipid peroxidation.

**Table 1: Showing Effects on Testicular Oxidative Stress Markers**

| Groups                       | Control                   | Arsenic                   | Extract only              | As + 200 mg/kg            | As + 400 mg/kg            |
|------------------------------|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|
| <b>SOD (U/mg protein)</b>    | 18.42 ± 0.56 <sup>a</sup> | 9.37 ± 0.48 <sup>c</sup>  | 19.01 ± 0.44 <sup>a</sup> | 14.28 ± 0.51 <sup>b</sup> | 17.36 ± 0.47 <sup>a</sup> |
| <b>CAT (U/mg protein)</b>    | 24.15 ± 0.72 <sup>a</sup> | 11.62 ± 0.63 <sup>c</sup> | 25.03 ± 0.68 <sup>a</sup> | 18.74 ± 0.59 <sup>b</sup> | 22.80 ± 0.61 <sup>a</sup> |
| <b>GSH (μmol/mg protein)</b> | 8.21 ± 0.31 <sup>a</sup>  | 3.74 ± 0.22 <sup>c</sup>  | 8.43 ± 0.29 <sup>a</sup>  | 6.02 ± 0.25 <sup>b</sup>  | 7.52 ± 0.28 <sup>a</sup>  |
| <b>MDA (nmol/mg protein)</b> | 2.11 ± 0.09 <sup>a</sup>  | 6.85 ± 0.18 <sup>c</sup>  | 2.03 ± 0.07 <sup>a</sup>  | 4.01 ± 0.14 <sup>b</sup>  | 2.68 ± 0.11 <sup>a</sup>  |

Values are Mean ± SEM (n = 7).

Different superscripts (a–c) across columns indicate significant difference at  $p < 0.05$ .

### 3.2 Effect on Serum Testosterone Levels

A significant decline in serum testosterone concentration was observed following arsenic exposure when compared with the control group. This reduction indicates impairment of testicular endocrine function, particularly disruption of Leydig cell steroidogenic activity.

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Treatment with *Annona muricata* extract significantly improved testosterone levels in arsenic-exposed animals. The low-dose treatment group exhibited moderate recovery of hormonal function, whereas the high-dose treatment group demonstrated testosterone concentrations that closely approximated those of the control animals. Furthermore, rats treated with the extract alone showed no evidence of hormonal suppression and maintained testosterone levels within the normal physiological range.

These findings suggest that *Annona muricata* possesses the capacity to preserve testicular steroidogenesis and mitigate arsenic-induced endocrine dysfunction.

**Table 2: Showing Effects on Serum Testosterone**

| Group                       | Control                  | Arsenic                  | Extract only             | As + 200 mg/kg           | As + 400 mg/kg           |
|-----------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|
| <b>Testosterone (ng/mL)</b> | 5.82 ± 0.21 <sup>a</sup> | 2.34 ± 0.15 <sup>c</sup> | 6.04 ± 0.19 <sup>a</sup> | 3.97 ± 0.18 <sup>b</sup> | 5.21 ± 0.22 <sup>a</sup> |

Values are Mean ± SEM (n = 7).

Different superscripts indicate statistical significance at p < 0.05.

### 3.3 Effect on Sperm Parameters

Arsenic administration produced significant impairment of sperm quality parameters. Animals exposed to arsenic alone exhibited substantial reductions in sperm count and progressive motility, accompanied by a marked increase in morphologically abnormal spermatozoa. These findings indicate severe disruption of spermatogenic processes and sperm maturation.

Co-administration of *Annona muricata* extract resulted in significant improvement in all evaluated sperm indices. The 200 mg/kg treatment group demonstrated partial restoration of sperm count and motility, together with a reduction in abnormal sperm forms. Greater improvement was observed in animals receiving 400 mg/kg extract, where sperm quality parameters approached values recorded in the control group.

The extract-only group maintained normal sperm characteristics throughout the experimental period, suggesting that the plant extract did not exert deleterious effects on male reproductive function. Overall, these results demonstrate a protective influence of *Annona muricata* against arsenic-induced deterioration of sperm quality.

**Table 3: Effect on Sperm Parameters**

| Group                                   | Control                   | Arsenic                   | Extract only              | As + 200 mg/kg            | As + 400 mg/kg            |
|-----------------------------------------|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|
| <b>Sperm Count (×10<sup>6</sup>/mL)</b> | 72.41 ± 2.11 <sup>a</sup> | 38.75 ± 1.96 <sup>c</sup> | 74.18 ± 2.34 <sup>a</sup> | 56.83 ± 2.08 <sup>b</sup> | 68.92 ± 2.15 <sup>a</sup> |

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**Motility (%)**             $85.32 \pm 1.78^a$      $41.84 \pm 2.03^c$      $87.15 \pm 1.69^a$      $63.42 \pm 1.85^b$      $79.61 \pm 1.72^a$

|                                |                   |                    |                   |                    |                   |
|--------------------------------|-------------------|--------------------|-------------------|--------------------|-------------------|
| <b>Abnormal Morphology (%)</b> | $6.41 \pm 0.34^a$ | $21.67 \pm 0.91^c$ | $5.92 \pm 0.28^a$ | $13.28 \pm 0.67^b$ | $8.74 \pm 0.41^a$ |
|--------------------------------|-------------------|--------------------|-------------------|--------------------|-------------------|

Values are Mean  $\pm$  SEM (n = 6).

Different superscripts indicate significant difference at  $p < 0.05$ .

### 3.4 Histological Findings

Histological examination corroborated the biochemical and hormonal findings. Arsenic exposure resulted in degeneration of seminiferous tubules, spermatogenic arrest, necrosis, cellular disorganization, and reduced Leydig cell population. Conversely, animals treated with *Annona muricata* demonstrated progressive restoration of testicular architecture, particularly at the 400 mg/kg dose where seminiferous tubules appeared nearly normal with active spermatogenesis.

**Control Group (Plate 1):** Animals in the control group displayed normal testicular histoarchitecture characterized by well-organized seminiferous tubules, intact germinal epithelium, abundant spermatogenic cells at various developmental stages, and densely populated luminal spermatozoa. Interstitial spaces contained morphologically normal Leydig cells without evidence of inflammation or vascular abnormalities.

**Arsenic-induced Group (Plate 2):** Testicular sections from arsenic-exposed animals exhibited severe histopathological alterations. These included degeneration and distortion of seminiferous tubules, depletion of germinal epithelial cells, vacuolar degeneration, spermatogenic arrest, and extensive cellular disorganization. Reduced Leydig cell density, vascular congestion, and areas of necrotic change were also observed. Collectively, these findings indicate profound impairment of testicular structure and function following arsenic exposure.

**Extract-only Group (Plate 3):** The extract-only group maintained normal testicular morphology comparable to that of the control animals. Seminiferous tubules remained structurally intact with active spermatogenesis and normal interstitial cell populations. No pathological abnormalities were observed.

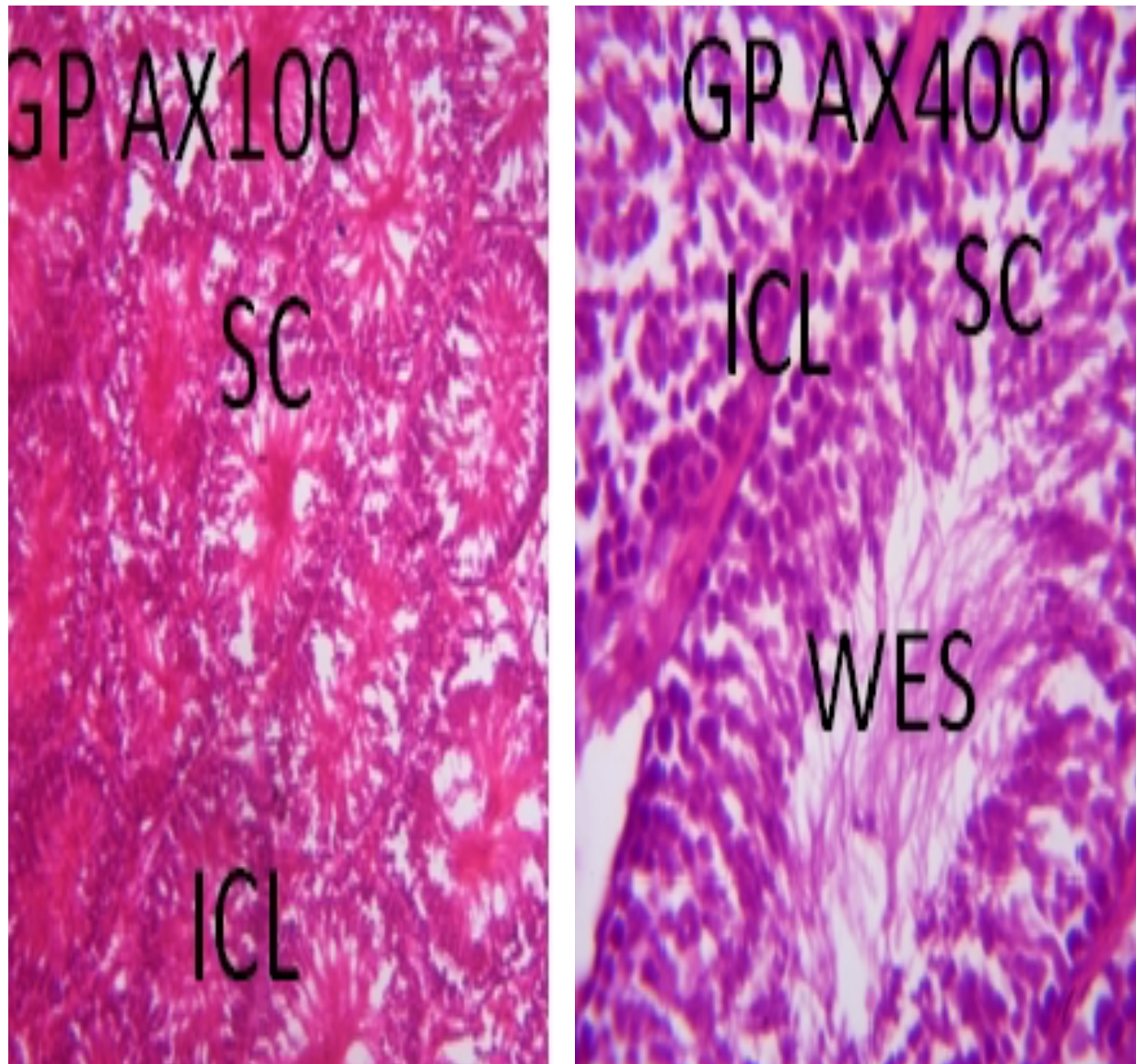
**Arsenic + 200 mg/kg Extract (Plate 4):** Animals receiving arsenic and 200 mg/kg of *Annona muricata* extract demonstrated moderate histological recovery. Seminiferous tubules showed partial restoration of architecture with re-establishment of several spermatogenic layers. Although mild degenerative changes persisted, overall tissue integrity was markedly improved relative to the arsenic-only group.

**Arsenic + 400 mg/kg Extract (Plate 5):** The high-dose treatment group exhibited substantial restoration of testicular architecture. Seminiferous tubules appeared largely normal with active spermatogenesis and increased luminal sperm density. Leydig cell morphology was preserved, and only minimal residual pathological changes were evident. Histological recovery in this group closely resembled that observed in the control animals.

### **3.5 The integrated Summary of the experimental Findings**

Arsenic exposure resulted in significant oxidative stress, suppression of testosterone production, deterioration of sperm quality, and extensive histological damage to testicular tissue. Administration of ethanolic leaf extract of *Annona muricata* effectively ameliorated these toxic effects in a dose-dependent manner. The highest dose tested (400 mg/kg) produced the greatest degree of biochemical, hormonal, spermatological, and histological recovery, indicating substantial protective activity against arsenic-induced reproductive injury.

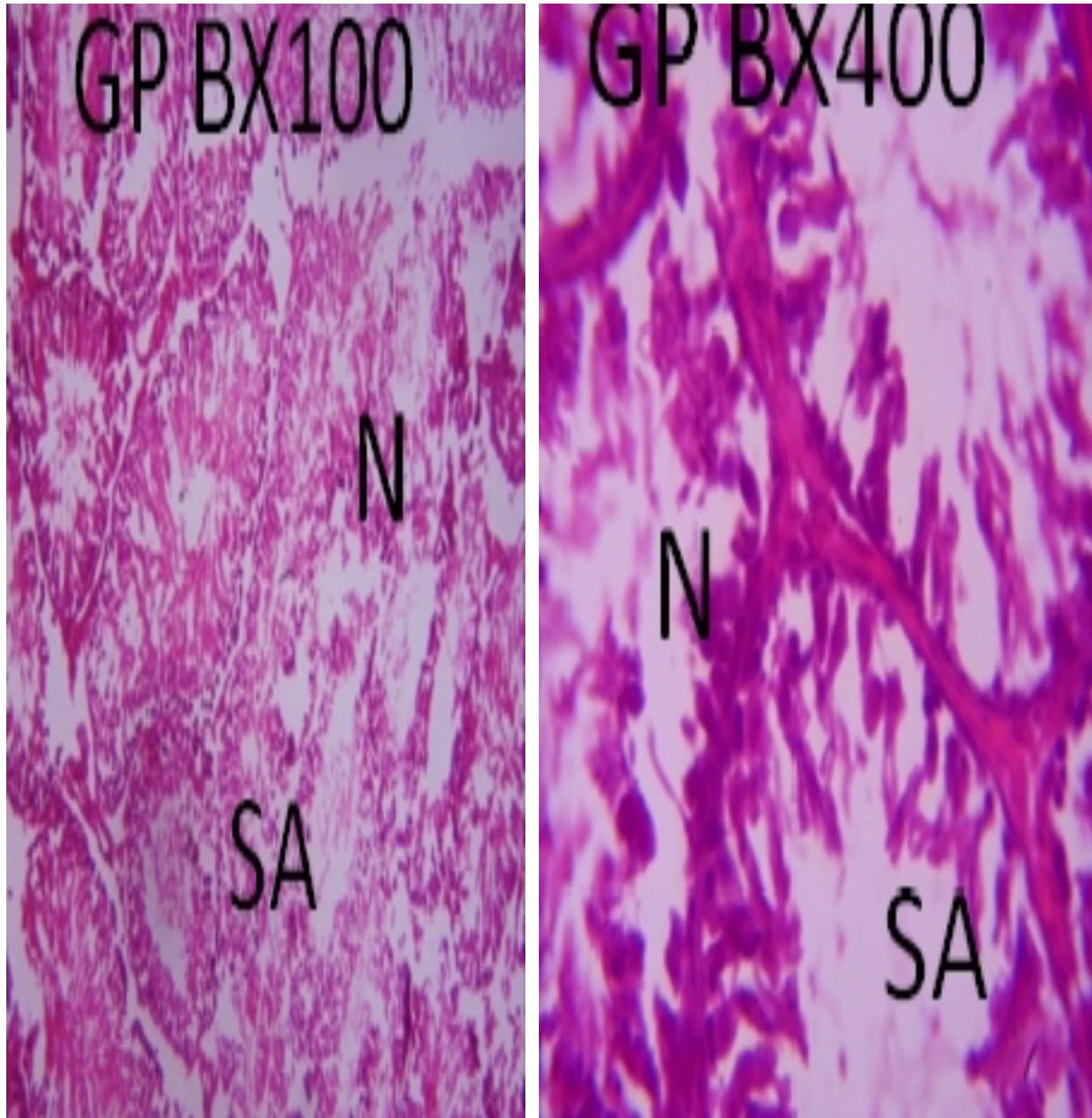
Control Group {A}



**Plate 1:** Photomicrographs of group A (Control) section of the testes (X100/X400 (H/E) shows normal testicular architecture with active seminiferous tubules that are lined with interstitial cells of the Leydig (ICL), Sertoli cells (SC) and well enhanced spermatogenesis (WES).

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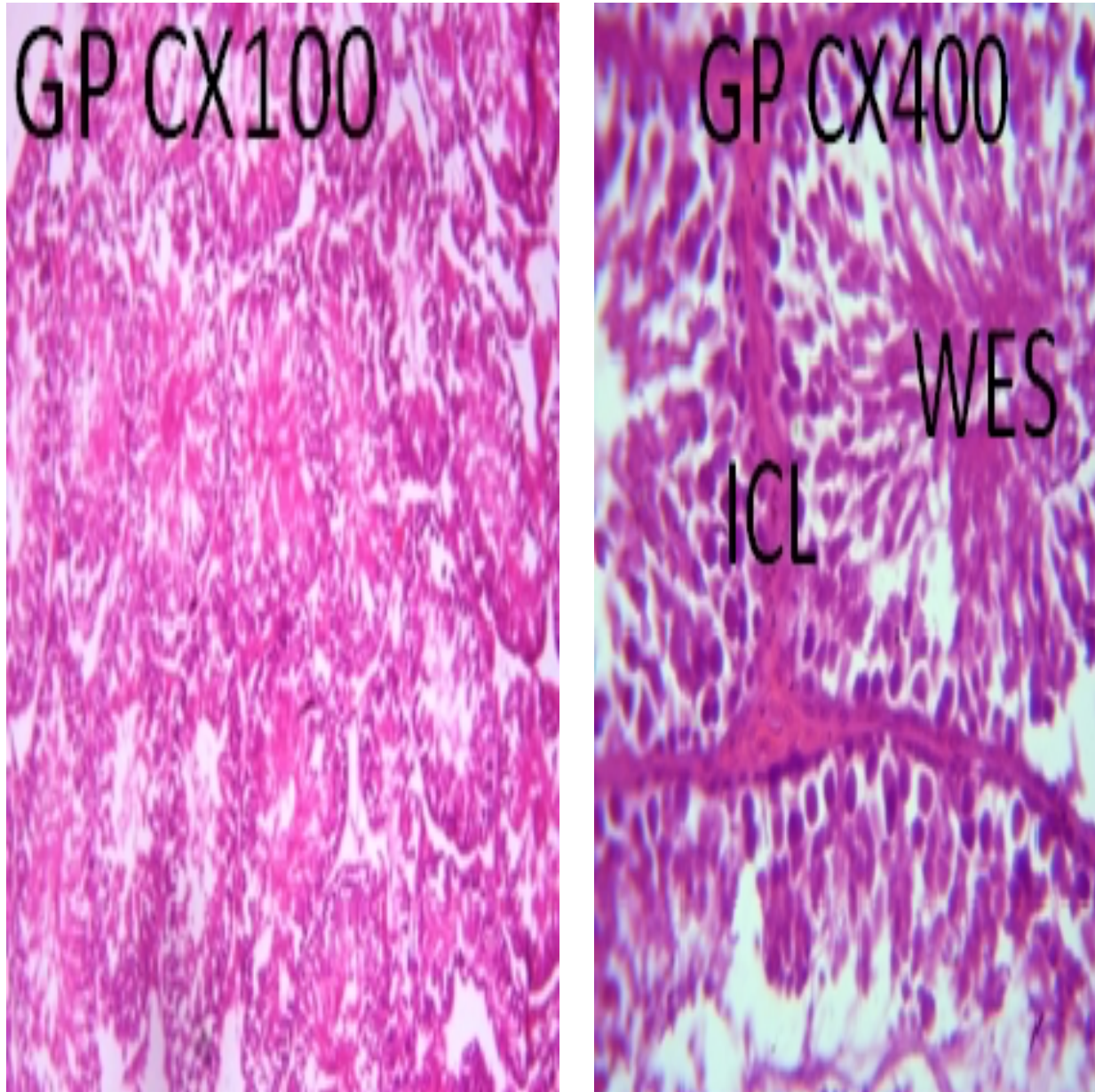
**Arsenic induced Group (B)**



**Plate 2:**Photomicrograph of group B sections of the testis induced with Arsenic (X100/X400(H/E)) shows severe degeneration of the testicular tissue with severe spermatogenic arrest (SA) and severe necrosis (N) of the testicular cells.

**Extract Only Group**

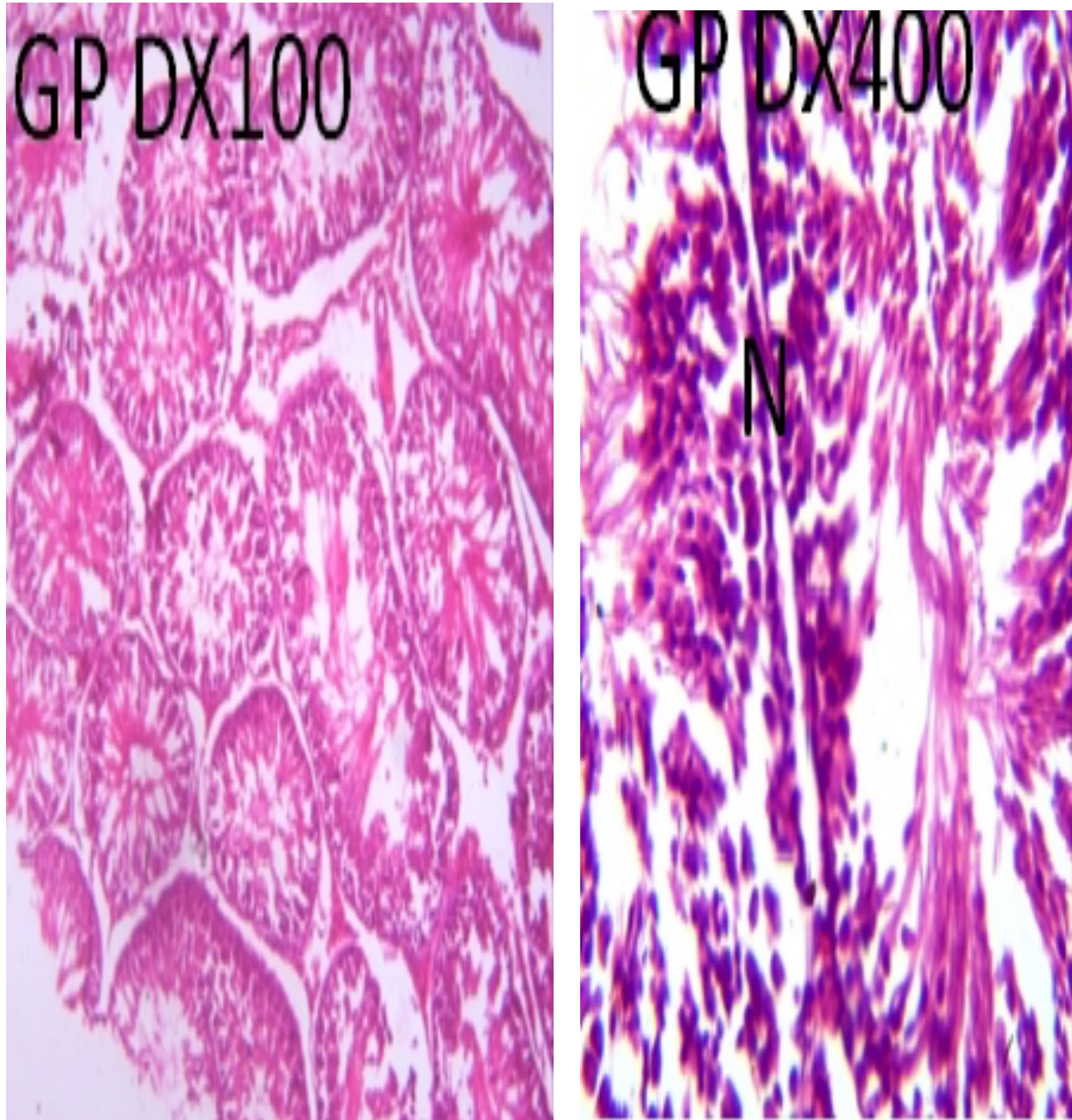
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**Plate 3:**Photomicrograph (100x400) (H/E) of control group C section of testis administered with 100mg/kg of the extract shows testicular architecture with active seminiferous tubules lined with distorted interstitial cells of Leydig (ICL) with Sertoli cell (SC) and well enhanced spermatogenesis (WES).

**Arsenic-induced + 200 mg/kg Extract**

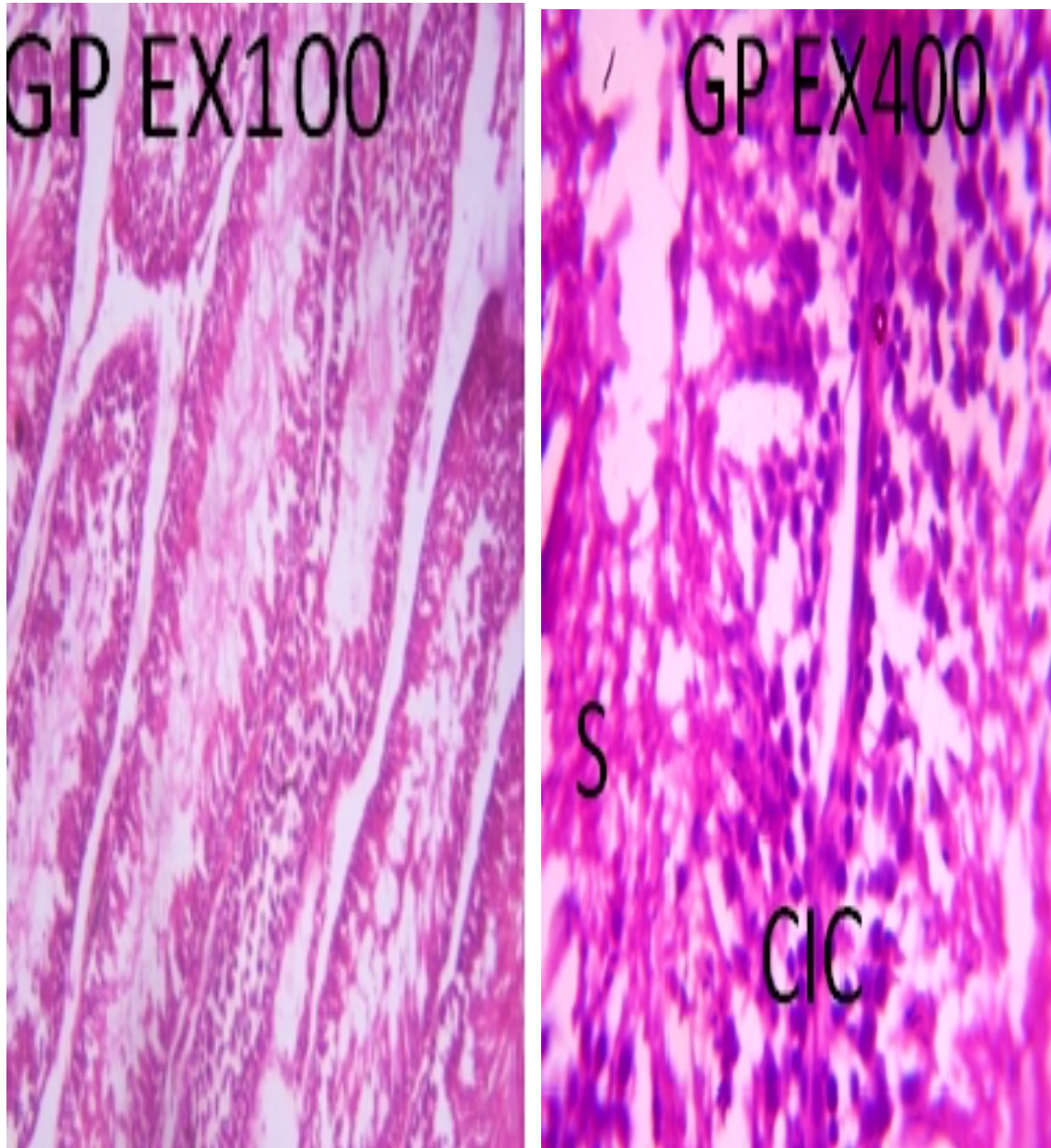
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**Plate 4:**Photomicrograph (X100/X400(H/E) of group D section of the testis induced with Arsenic and treated with 200mg/kg extract of *Annona muricata* shows moderate response with mild necrosis (N) of the testicular cells.

**Arsenic-induced + 400 mg/kg Extract**

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**Plate 5:**Photomicrograph (100/X400(H/E) of group E section of the testis induced Arsenic and treated with 400mg/kg of *Annona muricata* extract shows moderate response with moderately enhanced spermatogenesis (S) and mild cluster of inflammatory cells (CIC).

#### **4. DISCUSSION**

The present study demonstrates that arsenic exposure induces profound reproductive toxicity characterized by oxidative stress, endocrine dysfunction, impaired sperm quality, and histopathological alterations. These findings agree with previous reports that identified oxidative stress as a major mechanism of arsenic-induced reproductive damage (Flora, 2011; Chandra et al., 2013).

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The significant increase in malondialdehyde levels observed following arsenic exposure indicates enhanced lipid peroxidation and membrane damage. Similar findings have been reported in experimental models of arsenic toxicity where excessive ROS generation overwhelmed endogenous antioxidant defenses (Flora, 2011; Valko et al., 2007).

The testes are particularly susceptible to oxidative injury because of their high metabolic activity and elevated content of polyunsaturated fatty acids. Oxidative stress-induced damage may impair spermatogenesis and sperm function, leading to infertility (Aitken & Roman, 2008).

Administration of *Annona muricata* extract significantly improved antioxidant enzyme activities and reduced lipid peroxidation. This observation corroborates previous studies demonstrating the antioxidant and cytoprotective activities of *A. muricata* leaf extracts (Adewole et al., 2011; Acésio et al., 2017; Oyeleke et al., 2020; Moghadamtousi et al., 2015).

The restoration of serum testosterone levels following treatment with *Annona muricata* suggests preservation of Leydig-cell function and steroidogenic activity. Similar endocrine-protective effects have been reported in studies investigating the reproductive benefits of antioxidant-rich medicinal plants (Moghadamtousi et al., 2015).

The significant improvements in sperm count, motility, and morphology observed in extract-treated animals may be attributed to reduced oxidative stress and enhanced testosterone biosynthesis. These findings agree with reports demonstrating that antioxidant phytochemicals protect germ cells from ROS-mediated injury and improve reproductive performance (Aitken & Roman, 2008; Oyeleke et al., 2020).

The histological restoration observed in treated animals further confirms the protective role of *Annona muricata*. Similar tissue-protective effects have been documented in experimental studies evaluating the antioxidant and anti-inflammatory properties of the plant (Adewole et al., 2011; Coria-Téllez et al., 2018)..

## **Study Limitations**

Although the present study demonstrated significant protective effects of *Annona muricata* against arsenic-induced reproductive toxicity, certain limitations should be acknowledged. Molecular biomarkers associated with oxidative stress, apoptosis, inflammation, and steroidogenesis were not investigated. Furthermore, fertility indices such as mating performance, pregnancy outcomes, and offspring viability were not evaluated. Future studies incorporating gene expression analyses, immunohistochemical assessments, and reproductive performance testing would provide deeper mechanistic insights into the protective actions of the plant extract.

## **5. CONCLUSION**

The findings of the present investigation demonstrate that chronic arsenic exposure induces severe reproductive toxicity in male Wistar rats through mechanisms involving oxidative stress, hormonal disruption, impaired spermatogenesis, and structural degeneration of testicular tissue. Arsenic administration significantly increased lipid peroxidation while suppressing endogenous antioxidant defenses, resulting in reduced testosterone production, poor sperm quality, and marked histopathological abnormalities.

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Treatment with ethanolic leaf extract of *Annona muricata* effectively ameliorated these adverse effects in a dose-dependent manner. The extract restored antioxidant enzyme activities, reduced oxidative damage, improved reproductive hormone levels, enhanced sperm characteristics, and preserved normal testicular histoarchitecture. These protective effects are likely attributable to the synergistic actions of bioactive phytochemicals present within the plant, including flavonoids, phenolic compounds, tannins, and alkaloids.

The results suggest that *Annona muricata* possesses considerable phytotherapeutic potential for the prevention and management of arsenic-induced reproductive dysfunction. Given the increasing global burden of environmental toxicant exposure, natural products with antioxidant and cytoprotective properties may provide valuable complementary approaches for safeguarding male reproductive health. Further investigations involving molecular mechanisms, reproductive outcome studies, and clinical validation are warranted to establish its translational relevance and therapeutic applicability in human populations.

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## Conflict of Interest

The authors guarantee responsibility for all the data published in this manuscript. The authors confirm the absence of a conflict of interest and the absence of their financial interest in conducting this research and articulating this manuscript. This manuscript is extracted and written from an original research work and has never been published, neither is it under consideration for publication elsewhere.

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