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Original Research Article

Antioxidant Properties of *Parquetina nigrescens* Methanol Leaf Extract Ameliorates Arsenic-Induced Reproductive Functions Alteration in Male Wistar Rats

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ABSTRACT

This study investigated the effects of *Parquetina nigrescens* (PN) methanol leaf extract on oxidative stress-induced reproductive toxicity in male Wistar rats treated with arsenic trioxide (As). Arsenic is a major water pollutant noted for causing male reproductive toxicity. *Parquetina nigrescens* is used in traditional medicine for the management of numerous diseases, its leaf is credited with having antioxidant capacities. Forty male Wistar rats (150-180g) were randomly distributed into eight groups: 1 (control), 2 (As: 3mg/kg), 3, 4, 5 (250mg/kg PN, 500mg/kg PN and 1000mg/kg PN), 6, 7, and 8 (As + 250 mg/kg PN, As + 500mg/kg PN, As + 1000mg/kg PN). Oral administration was within 54 days. Spermatozoa from caudal epididymis were assessed for count, motility, progressive motility, viability and percentage of normal morphology. Serum testosterone concentration, testicular tissue concentration of malondialdehyde (MDA), superoxide dismutase (SOD) and glutathione peroxidase activities were assessed. Sperm count, viability and percentage of normal morphology were significantly reduced ($p<0.05$) in As group but improved in As groups co-administered 250, 500, and 1000 mg/kg PN. Testosterone level was improved in 1000 mg/kg PN and As + 250mg/kg PN in relation to the control and As group. The MDA was significantly increased while SOD declined ($p<0.05$) in group As. The SOD increased in groups administered As + 250mg/kg PN, As + 500mg/kg PN, and As + 1000 mg/kg PN. *Parquetina nigrescens* leaf extract ameliorated arsenic trioxide-induced sperm parameters and testosterone alteration via preventing oxidative stress in male Wistar rats.

Keywords: Arsenic trioxide, *Parquetina nigrescens* (PN), Sperm parameters, Oxidative stress and testicular Histology.

Introduction

Arsenic possesses the characteristics of non-metals and metals. For this reason, it is also known as a metalloid. In Africa and Europe, it is a major pollutant washed into different water sources via burning of nonferrous metals, casting, and mining.[1-3] Exposure to arsenic above 1.0×10^{-3} ppm for 8 hours was connected with a high hazard of oxidative impairment to male reproductive organs.[4] It also reduced stem cells spermatogonia, wider interstitial cells, Sertoli cells, and the damage of seminiferous tubules, leading to testicular weight loss, reduced testosterone level, sperm count, motility and genotoxicity.[5] *Parquetina nigrescens* (Afzel.) Bullock (Periplocaceae) is predominant in the forests of West Africa and thrives better in tropical weather conditions.[6] The Hausas and the Yoruba of Nigeria call it Kwànkwánín tsa tsumbe and Ogbo.[7]

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Also, *Parquetina nigrescens* is typically used in East and West Africa to enhance fertility in men and the treatment of insanity, diarrhoea, gonorrhoea, worm infections, fever, general body aches and menstrual disorders. [8-12] It has been reported that stomach aches and snake bites can be treated with the *Parquetina nigrescens* root decoction.[12] An *in-vitro* antioxidant assay conducted on the methanolic extract derived from *Parquetina nigrescens* (PN) leaf indicated that it contains flavonoids, phenols and has free radical scavenging effects against azino-bis-3-ethylbenzthiazoline-6-sulphonic acid and 2,2-Diphenyl-1-picrylhydrazyl hydrate radical; it also has metal chelating ability.[13-14] *Parquetina nigrescens* is also reported to have anti-diabetic and haematinic activities,[15] antimicrobial and gastro-protective properties,[16] cardiotonic sympathetic activities and hypoglycaemic effects.[17,7] Oxidative stress (OS) is a known key factor connected to arsenic-induced sperm cytotoxicity.[18] The mechanisms linked to arsenic-induced cytotoxicity are directly or indirectly driven by alteration in reactive oxygen species leading to OS.[19] Arsenic acts on the male reproductive system to inhibit steroidogenesis by hindering enzymes accountable for it, generating free radical,[20] oxidating spermatozoa DNA,[21] as well as inducing apoptosis.[22] Consequently, it is important that strategies to prevent or cure arsenic-incited toxicity in the male reproductive system ought to be investigated. The *Parquetina nigrescens* leaf has been credited with antioxidant capacity and has been used in experimental research to treat erectile dysfunction and low reproductive hormones induced with paroxetine hydrochloride, which is a serotonin-specific reuptake inhibitor (SSRI).[11] The actions of PN in mitigating male reproductive toxicity caused by exposure to arsenic are yet to be elucidated. This study investigated the antioxidant properties of *Parquetina nigrescens* methanol leaf extract on arsenic-induced reproductive functions alteration in male Wistar rats.

Materials and Methods

Plant collection
The *Parquetina nigrescens* leaf was harvested and authenticated (FHI: 109785) in the University of Ibadan campus, Oyo State, South West Nigeria and the Forestry Research Institute of Nigeria, Jericho, Ibadan, Oyo State, Nigeria respectively.

Method of extraction
Harvested leaves air dried within 6 weeks and thereafter pulverised. The powder (1100 g) was subjected to the Soxhlet method of extraction using absolute methanol as solvent.[23-14] The extract derived was freeze-dried, and a powdered form that weighed 104 g and known as PN was obtained and kept in a fridge.

Experimental design
A total of 40 male Wistar rats were distributed into 8 groups. The first two groups were the control (3 mL/kg of tap water) and arsenic (3 mg/kg As), three, four and five were administered 250 mg/kg PN, 500 mg/kg PN, and 1,000 mg/kg of PN respectively, while six, seven and eight were administered As + 250 mg/kg PN, As + 500 mg/kg PN, and As + 1,000 mg/kg PN.

Arsenic trioxide as well as PN were orally administered for 54 days. The dosage of As_2O_3 used was previously reported.[20] The PN was dissolved in 10% tween 80. Ethical approval for the study was obtained (UI-ACUREC/18/0067) from the University of Ibadan Animal Care and Use Research Ethics Committee and the guidelines for the care and use of laboratory animals were complied with.

Sacrifice and collection of organs
On day 55, intraperitoneal injection of 40 mg/kg sodium thiopental was administerd to sedate the animals.[24] Spermatozoa were obtained from the caudal epididymis and the testes were harvested, weighed and used for biochemical assays and histoarchitectural investigation.

Sperm analysis
Count and motility of spermatozoa: The epididymis was identified, cut at the caudal part and rinsed using two millilitres of phosphate buffer solution (PBS). A Neubauer's counting chamber was loaded with a drop of PBS containing spermatozoa. The counting and motility of sperm were done with a computer-assisted sperm analyser (JH-6004 CASA).

Sperm viability: A single drop of sperm obtained from epididymis was added to 0.5% eosin-nigrosin on a slide and examined microscopically using Olympus Binocular Biological microscope (Cx23) - a product of Wincom Company Ltd, Hunan, China. One hundred sperms were observed under the microscope, the non-viable and viable spermatozoa were recorded in percentage.

Sperm morphology**:** A drop of epididymal sperm on a glass slide was made into a thin smear and exposed to air. It was thereafter fixed in 95% and 50% ethanol for 15 minutes and 30 seconds respectively. The slide was then rinsed in distilled water for 30 seconds and stained with eosin, then rinsed with water and exposed to air. The slide was observed under the microscope and normal sperm morphology was expressed in percentage.[25]

Hormone assay: Cardiac puncture was used to obtain blood samples from the animals. The blood was centrifuged using Searchtech Instrument Electrical Centrifuge (80-3), China, at 3,000 revolutions per 15 minutes to obtain serum. Testosterone level was assayed by enzyme-linked immunosorbent assay (Calbiotech ELISA kits; Inc. California). The assay was conducted based on the guidelines provided in the kit.

Biochemical assay
Testis (1 g) was weighed and homogenized in 5 mL of phosphate buffer saline (PBS) with Teflon homogenizer and a supernatant was obtained. Malondialdehyde (MDA) was assayed by mixing supernatant (1.0 mL) and TCA-TBA-HCL (2.0 mL).[26] Superoxide dismutase (SOD) was determined by adding 2.5 mL of the carbonate buffer into 0.2 mL of the sample, after equilibration, 0.3 mL of adrenaline (0.3 mM) was introduced into the samples and reference tube.[27] Glutathione peroxidase was assayed by dispensing and thoroughly mixing 500 µL of phosphate buffer, 100 µL of sodium azide, 200 µL of reduced glutathione, 100 µL of hydrogen peroxide, 500 µL of sample and 600 µL of distilled water. The mixture was incubated and 0.5 mL of TCA was added and centrifuged. To 1 mL of the supernatant, 2 mL of dipotassium phosphate and 1 mL of 5'-5'-dithiobis-(2-dinitrobenzoic acid) were added.[28] Catalase activities were evaluated by mixing 0.5 mL of sample, 5.0 mL of 30 nM H_2O_2, 1.0 mL of 6M H_2SO_4, and 7.0 mL of 0.01M $KMnO_4$.[29] The method used was spectrophotometry, the absorbance of MDA, SOD, GPx and catalase were read at 535nm, 420nm, 420nm and 480nm respectively.

Histology of the Tissues
Testis was kept in Bouin's fluid for not less than 5 hours before being dehydrated, cleared, embedded in paraffin wax, trimmed, sectioned, and stained with hematoxylin & eosin. Thereafter, images of the histology were captured to observe morphological changes using 40× magnification.

Statistical Analysis
The data derived was subjected to analysis using one-way analysis of variance followed by Waller-Duncan's post-hoc test. This was done with IBM's Statistical Package for Social Sciences (SPSS), version 25.0. The group data was presented as mean ± standard error of mean. The p-value less than 0.05 was considered significant. The chats were plotted with GraphPad Prism 8.0.1 (244) version.

Results and Discussion

Sperm count (Figure 1) significantly reduced in As-reated group in relation to the control. Arsenic has been found to induce OS in testicular tissues, thus, causing a compromise of the integrity of spermatozoa's membrane, suppressing sperm production activities of the testis and reducing numbers of spermatozoa.[30-31] This effect was reversed in the As + 500 mg/kg PN group in relation to the animals treated with As. A significant increase in sperm count was seen in rats administered As + 250 mg/kg PN and As + 1,000 mg/kg PN in relation to the control and As groups. This outcome is likely due to an arsenic trioxide-induced decrease in sperm count. The PN-only group showed no difference in the number of spermatozoa. The antioxidant properties of PN reported in this study and previous study,[14] are likely to be responsible for the restoration of the spermatogenic functions and improved sperm count.

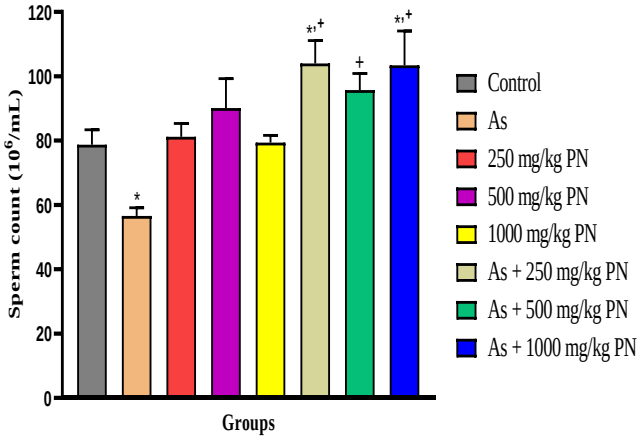

Figure 1: Effect of *Parquetina nigrescens* leaf extract on sperm count in arsenic trioxide-exposed male Wistar rats
Columns represent mean ± SEM, n = 5
*, $^+p < 0.05$ in relation to control and As respectively

An obvious decline in the percentage motility of sperms (Figure 2), progressive motility (Figure 3), as well as viability (Figure 4), was detected in As-treated group in relation to the control group, which affirms a similar study.[32] There is an abundant quantity of thiols in the spermatozoa that serve as binding sites for metals which reduce enzyme synthesis for motility.[18] Arsenic combines with thiols in sulfhydryl group proteins of sperm's nuclear chromatin and flagellum. Arsenic may utilize this mechanism to reduce the proportion of motile as well as the viable spermatozoa. A significant decline was observed in the percentage of viable spermatozoas in As + 1,000 mg/kg PN in relation to control. A significantly high percentage of sperm viability (Figure 4) was seen in As + 250 mg/kg PN and As + 500 mg/kg PN in relation to the As group. This indicated that dosages higher than 500 mg/kg may not have a positive effect on sperm viability.

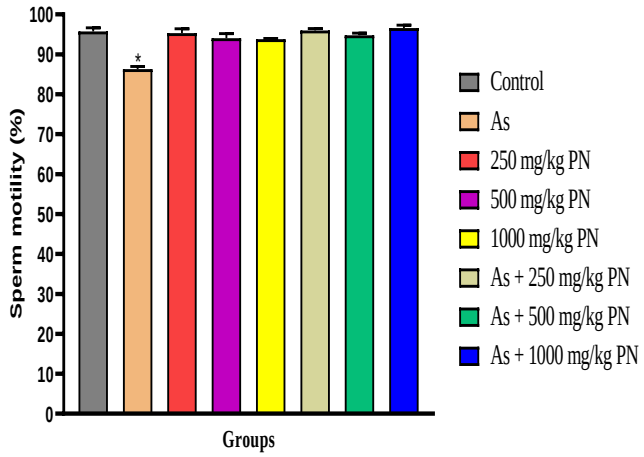

Figure 2: Effect of *Parquetina nigrescens* leaf extract on sperm motility in arsenic trioxide-exposed male Wistar rats
Columns represent mean ± SEM, n = 5
*p < 0.05 in relation to control

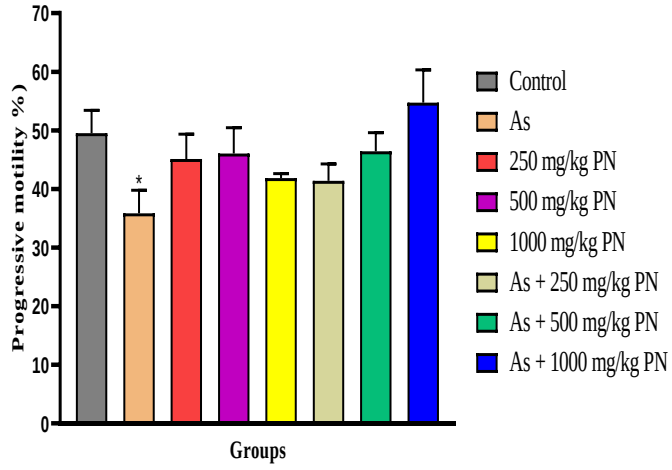

Figure 3: Effect of *Parquetina nigrescens* leaf extract on sperm progressive motility in arsenic trioxide-exposed male Wistar rats
Columns represent mean ± SEM, n = 5
*p < 0.05 in relation to control

The metal chelating property of PN reported in previous studies may be responsible for the prevention of As toxicity *via* the inhibition of its binding to thiol, thus improving enzyme synthesis required for sperm motility. The normal spermatozoa morphology expressed in percentage (Figure 5) decreased ($p<0.05$) in As. This is similar to a report which stated that As depressed spermatogenesis in rats' testis by reducing biosynthesis and release of luteinizing hormone and testosterone.[32] The groups treated with As and PN exhibited a significant rise in the normal spermatozoa morphology expressed in percentage in relations to As group. It was observed in this present study that PN improved testosterone concentration. Testosterone is required for normal spermatogenesis. Thus, it can be deduced that PN increased the percentage of normal sperm morphology through improving testosterone synthesis.

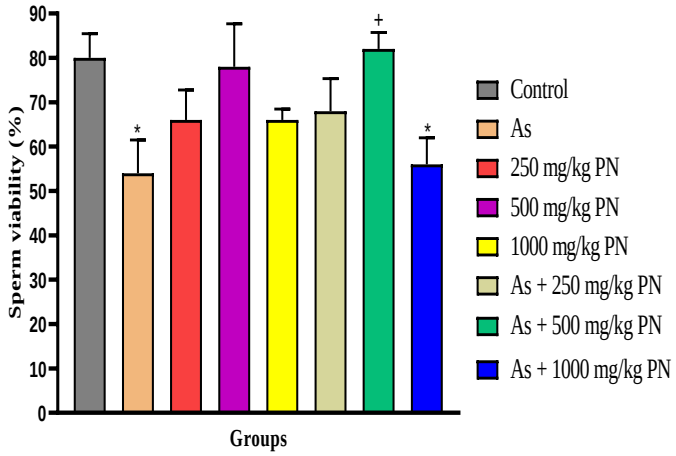

Figure 4: Effect of *Parquetina nigrescens* leaf extract on sperm viability in arsenic trioxide-exposed male Wistar rats
Columns represent mean ± SEM, n = 5
*, +p < 0.05 in relation to control and As respectively

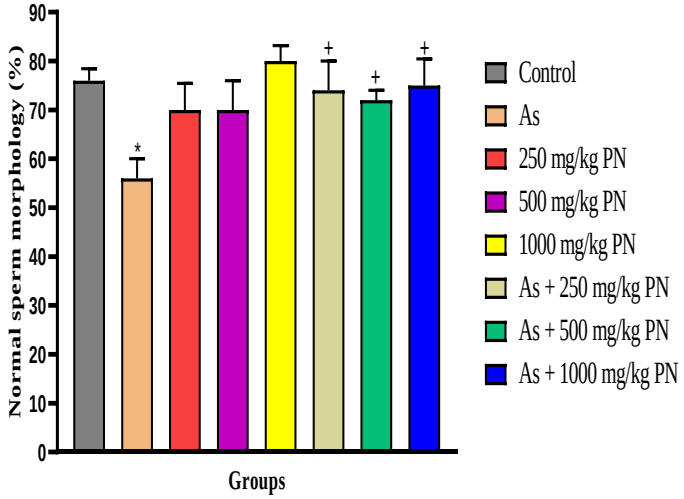

Figure 5: Effect of *Parquetina nigrescens* leaf extract on sperm morphology in arsenic trioxide-exposed male Wistar rats
Columns represent mean ± SEM, n =5
*, +p < 0.05 in relation to control and As respectively

Significant increases were recorded in testosterone levels of rats subjected to treatment with 1,000 mg/kg PN or with As + 250 mg/kg PN in relation to the control. An increase ($p<0.05$) was seen in testosterone level in the As + 250 mg/kg PN group relative to As group

(Figure 6). This is in agreement with a past study in which ethanol extract of *Parquetina nigrescens* roots improved testosterone concentration.[9] The improved testosterone level might have contributed to the improved sperm morphology, viability and count observed in this study. An increase ($p<0.05$) in testicular MDA concentration (Table 1) was seen in the rats administered with As. This concurs with a past study.[33] A decrease ($p<0.05$) in MDA concentration was seen in As + 250 mg/kg PN, As + 500 mg/kg PN, and As + 1000 mg/kg PN groups relative to the As group. The dose-dependent manner in which PN decreased MDA in these groups indicated that its potency against lipid peroxidation increases with increasing concentration. *Parquetina nigrescens* leaf extract has been reported to contain flavonoids, phenol, and is also ascribed with free radical scavenging activities, which may lessen OS.[13-14]

Table 1. Effect of PN on testicular malondialdehyde concentration, superoxide dismutase and catalase activities in As exposed male Wistar rats

Group	MDA level (nm/mg tissue)	SOD (U/mg)	Glutathione peroxidase (U/mg)	CAT (μ/mg tissue)
Control	11.66 ± 2.83	0.43 ± 0.05	0.29 ± 0.02	0.1 ± 0.009
As	30.65 ± 8.43*	0.66 ± 0.52	0.12 ± 0.01*	0.1 ± 0.007
250mg/kg PN	14.5± 1.41	0.44 ± 0.37	0.09 ± 0.06*	0.1 ± 0.001
500mg/kg PN	14.64 ± 3.76	2.77 ± 0.67	0.14 ± 0.001*	0.11 ± 0.002
1000mg/kg PN	9.5 ± 0.03	5.24 ± 1.03*	0.17 ± 0.05*	0.1 0.003
As + 250mg/kg PN	9.43 ± 2.86+	3.43 ± 0.5*,+	0.17 ± 0.05*	0.11 ± 0.002
As + 500mg/kg PN	4.28 ± 1.01+	2.95 ± 0.38*,+	0.23 ± 0.05+	0.09 ± 0.01
As + 1000mg/kg PN	3.13 ± 0.87+	0.91 ± 0.19	0.24 ± 0.05+	0.1 ± 0.002

Values represent mean ± SEM, n = 5. *, +$p < 0.05$ in relation to control and as respectively.

Thus, it can be deduced that PN increased the percentage of normal sperm morphology through improving testosterone synthesis. Significant increases were recorded in testosterone levels of rats subjected to treatment with 1,000 mg/kg PN or with As + 250 mg/kg PN in relation to the control. This composition of PN might be the cause of the observed decline in MDA concentration. This is likely to be responsible for the noted improvement in spermatozoa motility, count, viability and the morphology of groups co-administered with arsenic and PN in this study. A rise ($p<0.05$) in testicular SOD activity (Table 1) was seen in the 1,000 mg/kg PN group in relation to the control. Also, SOD activity was significantly higher in As + 250 mg/kg PN and As + 500 mg/kg PN with respect to the control and As groups respectively. The highest dosage might have stimulated the conservation of SOD synthesized within the testes due to the ability to dismutate free radicals, thereby making more SOD available. It is also likely that PN may stimulate SOD synthesis.

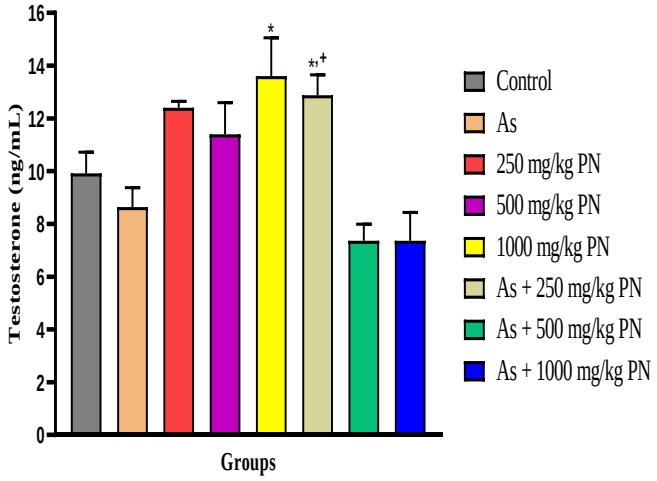

Figure 6: Effect of *Parquetina nigrescens* leaf extract on testosterone concentration in arsenic trioxide-exposed male Wistar rats
Columns represent mean ± SEM, n =5

*, +$p < 0.05$ in relation to control and as respectively

The effect of PN on SOD activities as presented in this report is in consonance with a past study in which melatonin, when co-administered with arsenic caused an increased activity of SOD, catalase and glutathione peroxidase.[34] It also corroborates the fact that there exists medicinal plants such as PN and *Sida corymbosa* leaves that has SOD activity-enhancing properties.[35] PN might have efficiently scavenged the free radicals promptly as was proven by the decline in MDA levels of the study groups, thereby, spearing the pro-oxidant and antioxidant balance.

GPx, the most predominant antioxidant enzyme in the testis normally detoxifies H_2O_2 in the mammalian mitochondria.[34] There was an observed reduction in GPx activity in the testis of As group (Table 1). This observation is in line with a study in which male Wistar rats were exposed to arsenic for 21 days.[31] Also, a decrease was seen in PN groups and As + 250 mg/kg PN group in relation to the control group. The enzyme activity increased in As + 500 mg/kg PN and As + 1,000 mg/kg PN in relation to the As group. The increase might have been induced by the negative impact of As in the presence of PN, which is proven to have antioxidant activities due to its chemical constituents. The increase in GPx activities in As + 500 and As + 1000 mg/kg groups was reflected in drastically reduced MDA concentration. The PN may mitigate against an increase in free radical generation caused by exposure to As as evidenced by decreased MDA, as well as an increase in GPx activity. The activity of catalase (Table 1) is similar in all the experimental groups. The testicular histology (Figure 7) of the As group presented a non-appearance of germ cell layers, empty and atrophied seminiferous tubules. This aligns with another study in which the administration of 50 ppm sodium-arsenate to C57BL/6 mice for 180 days resulted in the disorganization of the epithelial lining of the seminiferous tissues, diminished layers and detachment of spermatogenic cells, and consequently, sloughing off.[36] As already shown, As caused a great rise in testicular MDA level, thus, the spermatozoa and germ cells were exposed to oxidative damage which may have predisposed them to failure of maturation and death. The histologic appearance of the remaining treated groups looked normal in comparison to the control. The PN activities in protecting the architecture of the testis may be due to its ability to reduce MDA concentration, while improving SOD and

GPx activities as well as that of testosterone concentration, thus preventing oxidative stress that may adversely affect the steroidogenic function of the testis.

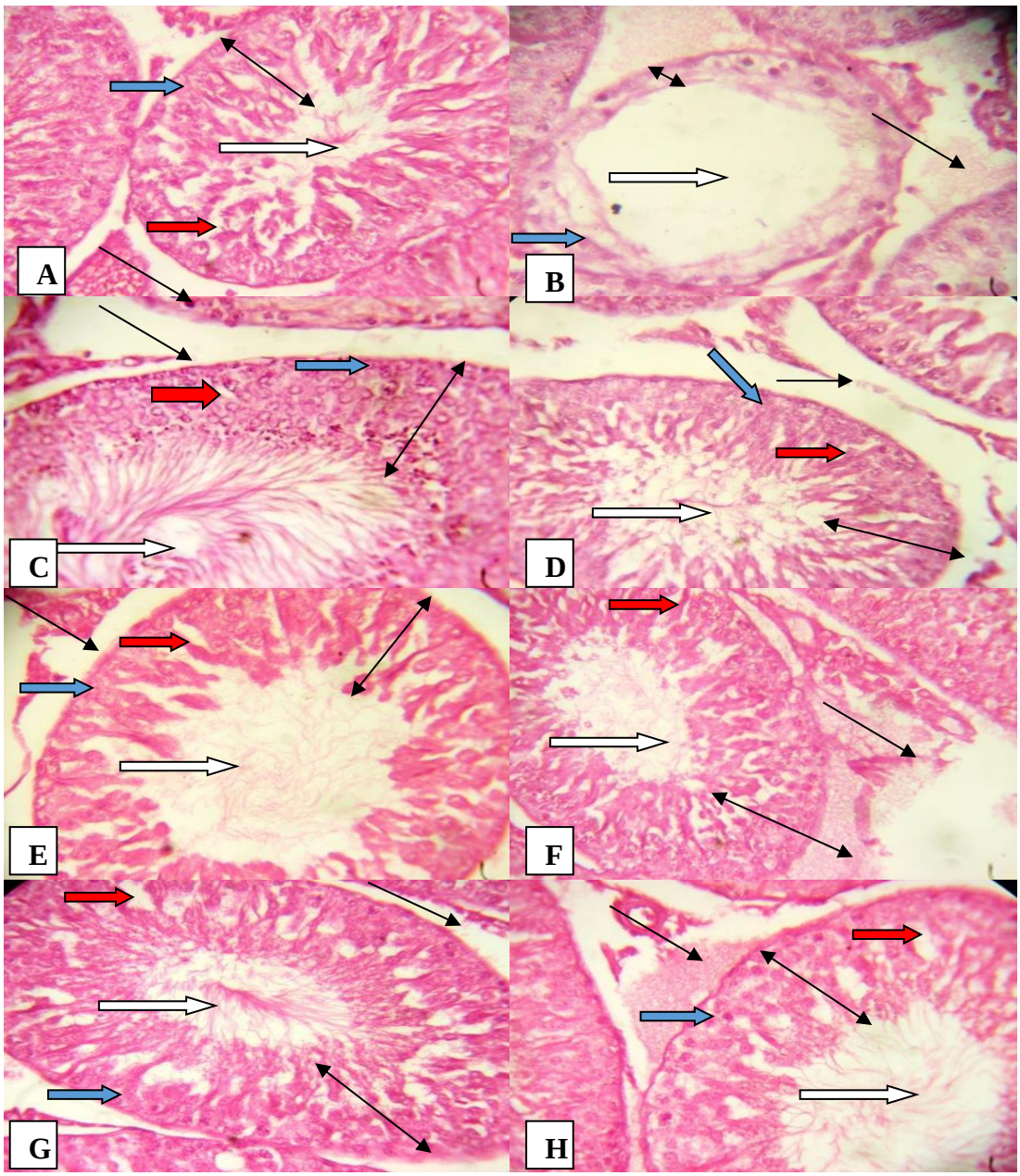

Figure 7: Photomicrograph of testicular sections from Wistar rats treated with *Parquetina nigrescens* leaf extract and arsenic. H&E stain (400×).

(A) Control, (B) As (C) 250 mg/kg PN, (D) 500 mg/kg PN, (E) 1,000 mg/kg PN, (F) As + 250 mg/kg PN, (G) As + 500 mg/kg PN, (H) As + 1,000 mg/kg PN.

Keys

White arrow	Seminiferous tubule lumen	Red arrow	Sertoli cells
Spanned arrow	Germ cell layer	**S**lender arrow	Interstitial space
Blue arrow	Spermatogonia cells		

Conclusion

Conclusively, Methanol extract of *Parquetina nigrescens* leaf demonstrated its potential to ameliorate low sperm count, low sperm viability, reduced percentage of normal sperm morphology, serum testosterone concentration and poor testicular histology induced by oral administration of arsenic trioxide via reduction of MDA concentration, elevation of SOD and GPx activities. The compounds present in PN should be isolated and studied to ascertain their effects on male reproduction, as well as their operational mechanisms.

Conflict of Interest

The authors declare that there is no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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