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PHARMACOLOGICAL EVALUATION OF STANDARDIZED EXTRACT OF *BLACK PEPPER* ON SODIUM ARSENITE-INDUCED REPRODUCTIVE TOXICITY IN EXPERIMENTAL RATS

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Abstract:

Background: *Sodium arsenite is an environmental toxicant capable of producing oxidative stress and impairment of male reproductive function. The present study investigated the protective effect of standardized* Piper nigrum *(black pepper) extract against sodium arsenite-induced reproductive toxicity in experimental rats.*

Methods: *Animals were randomly divided into six groups (n = 6): normal control, sodium arsenite control, CoQ10 (10 mg/kg), and standardized* P. nigrum *extract-treated groups receiving 50, 100, or 200 mg/kg. Sodium arsenite was administered orally at 10 mg/kg. Body weight and reproductive parameters were assessed, including absolute and relative weights of the testes, epididymis, and prostate, sperm count, sperm motility, dead and abnormal sperm, and serum testosterone. Testicular homogenates were evaluated for total protein, superoxide dismutase (SOD), reduced glutathione (GSH), malondialdehyde (MDA), and nitric oxide (NO). Testicular histopathology was also examined.*

Results: *Sodium arsenite produced significant reproductive and biochemical alterations, including reductions in body and reproductive-organ weights, sperm count, sperm motility, testosterone, SOD, GSH, and testicular protein, together with increases in dead and abnormal sperm, MDA, and NO. The 200 mg/kg dose of standardized black pepper extract significantly attenuated most of these changes compared with the sodium arsenite control group. Histopathological examination further demonstrated reduced inflammatory infiltration, necrosis, Leydig cell damage, and spermatogenic-cell vacuolation following treatment.*

Conclusion: *Standardized* P. nigrum *extract, particularly at 200 mg/kg, demonstrated protective activity against sodium arsenite-induced reproductive toxicity, potentially through attenuation of oxidative and nitrosative stress and preservation of testicular structure and reproductive function. Further mechanistic and safety studies are warranted.*

Keywords: Piper nigrum*; black pepper; sodium arsenite; reproductive toxicity; oxidative stress; sperm quality; testosterone; testis; SOD; GSH; MDA; nitric oxide.*

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INTRODCUTION:

Arsenic is a naturally occurring environmental metalloid that can enter the human body through contaminated drinking water, food, soil, and occupational exposure. Among its different chemical forms, inorganic arsenic compounds such as arsenite are of particular toxicological concern. Chronic or repeated exposure to arsenic has been associated with adverse effects involving multiple organ systems, including the nervous, cardiovascular, hepatic, renal, and reproductive systems. The male reproductive system is particularly vulnerable to toxicant-induced oxidative and cellular injury because normal spermatogenesis requires tightly regulated redox homeostasis and coordinated endocrine function.
Sodium arsenite is a widely used experimental toxicant for investigating arsenic-associated tissue injury. Exposure to sodium arsenite can disturb cellular redox balance and promote excessive generation of reactive oxygen species (ROS). Increased oxidative stress can initiate lipid peroxidation, alter cellular proteins and nucleic acids, impair mitochondrial function, and compromise cellular antioxidant defenses. In testicular tissue, these effects may interfere with the structural and functional integrity of seminiferous tissue and affect the processes required for normal sperm development and maturation. The present study similarly demonstrated substantial alterations in oxidative and reproductive parameters following sodium arsenite exposure, including increased testicular MDA and NO with depletion of SOD and GSH.

Arsenic-induced reproductive toxicity can manifest through several interconnected alterations, including impaired spermatogenesis, deterioration of sperm quality, changes in reproductive-organ weight, disruption of steroidogenic activity, and structural damage to the testes. Testosterone is particularly important for the maintenance of spermatogenesis and male reproductive function. Damage to testicular cells and disruption of steroidogenic processes may therefore result in reduced circulating testosterone and subsequent deterioration of reproductive performance. In the present experimental model, sodium arsenite exposure was associated with marked reductions in sperm count, sperm motility, and serum testosterone, together with reductions in testicular, epididymal, and prostate weights.

Oxidative stress is considered an important component of toxicant-mediated testicular injury because excessive ROS generation can overwhelm endogenous antioxidant systems. Superoxide dismutase and reduced glutathione constitute important components of cellular antioxidant defense, whereas increased MDA reflects enhanced lipid peroxidation and membrane damage. Nitric oxide and its reactive derivatives can additionally contribute to nitrosative stress under pathological conditions. Consequently, simultaneous evaluation of antioxidant status and oxidative/nitrosative stress markers provides useful information regarding the biochemical alterations associated with reproductive toxicity.

Natural products containing bioactive phytoconstituents have received considerable attention as potential sources of protective agents against chemically induced oxidative injury. *Piper nigrum* L. (Piperaceae), commonly known as black pepper, is an important culinary spice and medicinal plant traditionally used for various pharmacological purposes. Its biological activities have been associated particularly with piperine and other phenolic and volatile constituents. Piperine has been investigated for antioxidant and other cytoprotective activities and may influence several biochemical pathways involved in cellular stress responses. These properties provide a pharmacological rationale for evaluating standardized *P. nigrum* extract in experimental models of oxidative tissue injury.

In the present study, a standardized extract of *Piper nigrum* was investigated against sodium arsenite-induced reproductive toxicity in rats. The study assessed changes in body weight, reproductive-organ weights, sperm count and motility, dead and

abnormal sperm, serum testosterone, testicular protein, SOD, GSH, MDA, and NO, together with histopathological changes in testicular tissue. The protective response was evaluated at three doses of standardized black pepper extract (50, 100, and 200 mg/kg), with CoQ10 included as a reference treatment. The objective was to determine whether standardized *P. nigrum* extract could attenuate the biochemical, functional, and structural alterations associated with sodium arsenite-induced reproductive toxicity. The observed findings demonstrated particularly evident protective effects at 200 mg/kg, with improvements in several reproductive, antioxidant, and histopathological parameters.

MATERIALS AND METHODS:

MATERIALS:

The standardized extract of *Piper nigrum* (black pepper) was procured from Synthite Industries (P) Limited, India (Batch No. 2160034362/10). Sodium arsenite was used as the toxicant for induction of reproductive toxicity. Biochemical and antioxidant assay reagents, including adenosine triphosphate (ATP), NADPH, epinephrine, 3,4-dihydroxyphenylacetic acid, acetylcholine iodide, 1,1-diphenyl-2-picrylhydrazyl (DPPH), malondialdehyde bis-dimethyl acetal, 2-thiobarbituric acid (TBA), Folin–Ciocalteu reagent, Griess reagent, Bradford reagent, and potassium dichromate, were obtained from standard commercial suppliers. Analytical-grade sodium citrate dihydrate, potassium hydroxide, sodium hydroxide, sulphuric acid, hydrochloric acid, phosphoric acid, acetic acid, trichloroacetic acid, chloroform, ethanol, formaldehyde, boric acid, sodium chloride, potassium chloride, potassium phosphate salts, sodium phosphate salts, magnesium sulphate, sodium hydrogen carbonate, copper sulphate, ammonium molybdate, sulphanilamide, and hydrogen peroxide were procured from recognized chemical suppliers. Polysorbate 80, Triton X-100, bovine serum albumin, EDTA, aminoguanidine bicarbonate, and other analytical reagents required for biochemical estimations were obtained from certified laboratory suppliers. Anaesthetic ether was procured from Rachana Ether India Pvt. Ltd., Wani, Maharashtra, India. All chemicals and reagents were of analytical or laboratory grade and were stored under the conditions recommended by the respective manufacturers until use.

Animals:

Sprague Dawley rats weighing 180-200 gm were purchased from Global Bioresearch Solutions Private Limited, H No 251 Nhavi, Tal-Bhor, Dist-Pune. The animals were housed in polypropylene cages and maintained under the environmental condition of temperature 25±1 ºC and relative humidity of 45-55 % under a 12h light: 12 dark cycles. The animals had free access to food pellets (Nav Maharashtra Chakan oil mills Ltd., Pune) and water ad libitum. The Institutional Animal Ethics Committee (IAEC) of 2168/PO/Re/S/22/CPCSEA approved all the experimental protocols under the Committee for the Purpose of Control and Supervision of Experiment on Animals (CPCSEA). The protocol approval number is 2168/PO/Re/S/22/CPCSEA.

METHODS:

Preparation and Administration of Test and Reference Treatments

A freshly prepared aqueous suspension of standardized *Piper nigrum* (black pepper) extract was used for oral administration. The required quantity of extract was dispersed in distilled water, and the dose volume was adjusted according to the body weight of each experimental animal. Fresh preparations were made daily and maintained in airtight amber-colored containers at room temperature until administration. The standardized black pepper extract was administered orally at doses of 50, 100, and 200 mg/kg body weight.

Coenzyme Q10 (CoQ10) was used as the reference treatment. The contents of the CoQ10 soft gelatin capsules were dispersed in 1% sodium carboxymethylcellulose to prepare the dosing suspension. A fresh suspension was prepared on each treatment day, with the administered volume calculated according to individual animal body weight. CoQ10 was administered orally at a dose of 10 mg/kg body weight.

Experimental Animals and Induction of Sodium Arsenite-Induced Reproductive Toxicity

The experimental animals were randomly allocated into six groups, with six rats in each group. The normal group received distilled water as the vehicle. Reproductive toxicity was induced by oral administration of sodium arsenite (10 mg/kg) for two consecutive days. The reference-treatment group received CoQ10 (10 mg/kg), whereas the treatment groups received standardized P. nigrum extract at doses of 50, 100, and 200 mg/kg. The black pepper extract was administered orally according to the predetermined treatment schedule, with treatment continued throughout the experimental period. The experimental groups were designated as normal control, sodium arsenite control, CoQ10-treated, and low-, medium-, and high-dose black pepper extract-treated groups, respectively.

Table 1. Experimental grouping and treatment schedule

Group	Treatment
Group I	Vehicle/normal control
Group II	Sodium arsenite, 10 mg/kg
Group III	Sodium arsenite + CoQ10, 10 mg/kg
Group IV	Sodium arsenite + *P. nigrum* extract, 50 mg/kg
Group V	Sodium arsenite + *P. nigrum* extract, 100 mg/kg
Group VI	Sodium arsenite + *P. nigrum* extract, 200 mg/kg

Assessment of *In Vivo* Parameters

Body Weight

Individual body weights of the experimental animals were recorded using a calibrated animal weighing balance. Body weight was monitored throughout the experimental period and used for calculation of the appropriate dose volume for administration.

Estimation of Serum Testosterone

At the end of the experimental period, blood samples were collected from anesthetized animals by retro-orbital puncture. Blood samples were centrifuged at 7000 rpm for 15 min at 4°C, and the separated serum was collected for testosterone estimation. Serum testosterone concentration was determined using a commercially available rat testosterone immunoassay kit (MyBioSource, USA; Cat. No. MBS740121) according to the manufacturer's instructions. The assay was based on an enzyme immunoassay principle in which testosterone present in the sample was detected through antibody-mediated binding followed by an enzyme-substrate reaction. Absorbance was measured using a UV–visible spectrophotometer (Jasco V-530, Tokyo, Japan), and testosterone concentrations were calculated from the standard calibration curve. Results were expressed as ng/mL serum.

***Ex Vivo* Biochemical Evaluation**

Following completion of the experimental protocol, the animals were sacrificed according to the approved experimental procedure. The testes, epididymis, and prostate were rapidly excised, cleaned of adhering tissues, and processed for biochemical and histopathological investigations. Testicular tissue homogenates were prepared in 0.1 M Tris-HCl buffer (pH 7.4). The resulting supernatants were used for estimation of total protein, lipid peroxidation, superoxide dismutase (SOD), reduced glutathione (GSH), and nitric oxide (NO).

Estimation of Lipid Peroxidation

Lipid peroxidation was assessed by measuring malondialdehyde (MDA), a product of membrane lipid oxidation, using the thiobarbituric acid-reactive substances method. Tissue homogenate supernatant was treated with 10% trichloroacetic acid and maintained under chilled conditions to precipitate proteins. Following centrifugation, the clear supernatant was reacted with thiobarbituric acid and heated in a boiling water bath to develop the colored reaction product. After cooling, absorbance was recorded at 532 nm against an appropriate reagent blank. MDA concentrations were calculated using a standard curve and expressed as nM MDA/mg protein.

Estimation of Superoxide Dismutase Activity

SOD activity in the testicular homogenate was determined based on inhibition of epinephrine auto-oxidation. The tissue supernatant was treated with ice-cold ethanol and chloroform, followed by centrifugation to obtain the enzyme-containing supernatant. The reaction mixture consisted of the supernatant, carbonate buffer, and EDTA, and the reaction was initiated by addition of epinephrine. The change in absorbance was monitored at 480 nm. SOD activity was calculated based on the degree of inhibition of epinephrine oxidation and expressed as units/mg protein.

Estimation of Reduced Glutathione

Reduced glutathione (GSH) content was determined using the reaction of tissue sulfhydryl groups with 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB). Equal volumes of tissue supernatant and 20% trichloroacetic acid were mixed, followed by centrifugation. An aliquot of the resulting supernatant was reacted with DTNB and phosphate buffer. The intensity of the developed yellow-colored product was measured spectrophotometrically at 412 nm. GSH concentration was calculated from a standard glutathione calibration curve and expressed as µg GSH/mg protein.

Estimation of Nitric Oxide

Nitric oxide production was estimated indirectly by determining nitrite concentration using the Griess reaction. Tissue samples were processed to convert nitrate to nitrite, followed by reaction with sulfanilamide and N-(1-naphthyl)ethylenediamine to generate a colored azo compound. The absorbance of the reaction product was measured at 543 nm. Nitrite concentration was calculated from an appropriate standard curve and expressed relative to tissue protein content as µg/mg protein.

Estimation of Total Protein

Total protein concentration in the tissue homogenate was determined by the Lowry method, using bovine serum albumin as the reference standard. Appropriate aliquots of diluted tissue

samples were treated with alkaline copper reagent and subsequently with Folin–Ciocalteu phenol reagent. After development of the characteristic blue color, absorbance was measured at 660 nm against a reagent blank. Protein concentration was calculated from the BSA calibration curve and expressed as mg/g wet tissue.

Histopathological Examination

For histopathological assessment, representative testicular tissue samples were immediately collected and fixed in 10% formalin. The tissues were processed through graded alcohol, cleared with xylene, and embedded in paraffin wax. Sections approximately 5 µm thick were prepared and stained with hematoxylin and eosin. The stained sections were examined under a light microscope (Nikon E200) at appropriate magnification. Histological alterations, including inflammatory cell infiltration, vascular congestion, edema, degeneration, and necrotic changes, were evaluated qualitatively and compared among the experimental groups.

Statistical Analysis

All experimental data were subjected to statistical analysis using GraphPad Prism version 5.0 (GraphPad Software, San Diego, CA, USA). Results were expressed as mean ± standard error of the mean (SEM). Comparisons were made between the sodium arsenite-treated control group and the respective treatment groups to determine the effects of standardized P. nigrum extract and CoQ10. A probability value of $P < 0.05$ was considered statistically significant.

RESULTS:

Effect of Standardized Black pepper Extract on Body Weight

Sodium arsenite exposure produced a marked reduction in body weight compared with the normal control group. The vehicle-control animals showed a body weight of 217.50 ± 3.82 g compared with 281.30 ± 2.77 g in normal animals ($P < 0.001$). Coenzyme Q10 (10 mg/kg) substantially attenuated the arsenite-associated reduction, with a body weight of 274.30 ± 2.72 g. Among the black pepper-treated groups, the 50 and 100 mg/kg doses did not produce a statistically significant improvement, whereas the 200 mg/kg dose significantly increased body weight to 262.00 ± 2.72 g compared with the vehicle-control group ($P < 0.001$). These findings indicate that the higher dose of standardized black pepper extract was associated with attenuation of arsenite-induced body-weight loss.

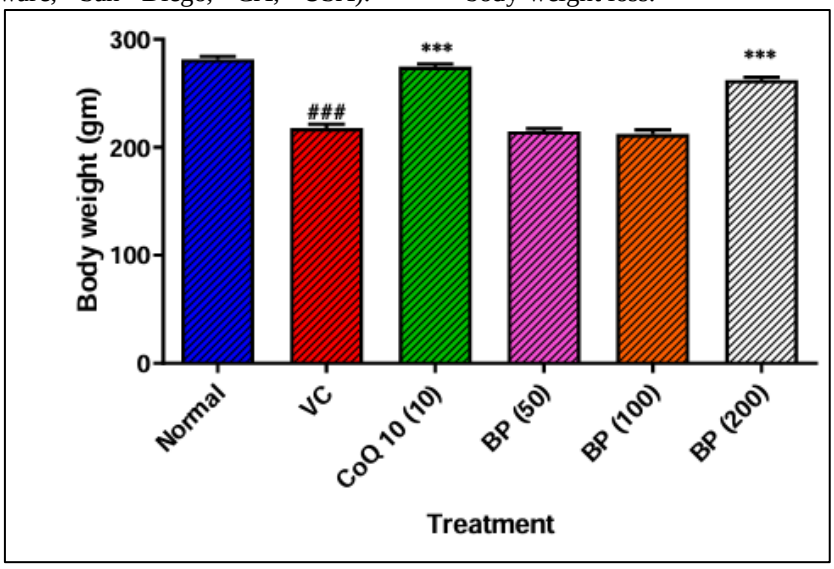

Figure 1: Effect of black pepper on arsenic-induced alteration in body weight

*Data were analyzed by One-Way ANOVA followed by Dunnett's. ###P < 0.001 as compared with normal group and ***P < 0.001 as compared with Vehicle Control group on respective days.*

Effect of Standardized Black pepper Extract on Testicular Weight

Sodium arsenite administration significantly reduced both absolute and relative testicular weights. Absolute testicular weight decreased from 1.78 ± 0.05 g in normal animals to 1.01 ± 0.05 g in the vehicle-control group ($P < 0.001$), while relative testicular weight decreased from 6.33 ± 0.21 × 10^{-3} to 4.66 ± 0.23 × 10^{-3}. CoQ10 treatment significantly attenuated these changes. Treatment with black pepper at 200 mg/kg significantly increased absolute testicular weight to 1.74 ± 0.03 g and relative testicular weight to 6.66 ± 0.17 × 10^{-3} compared with the vehicle-control group ($P < 0.001$). The 50 and 100 mg/kg doses did not demonstrate statistically significant changes in either parameter.

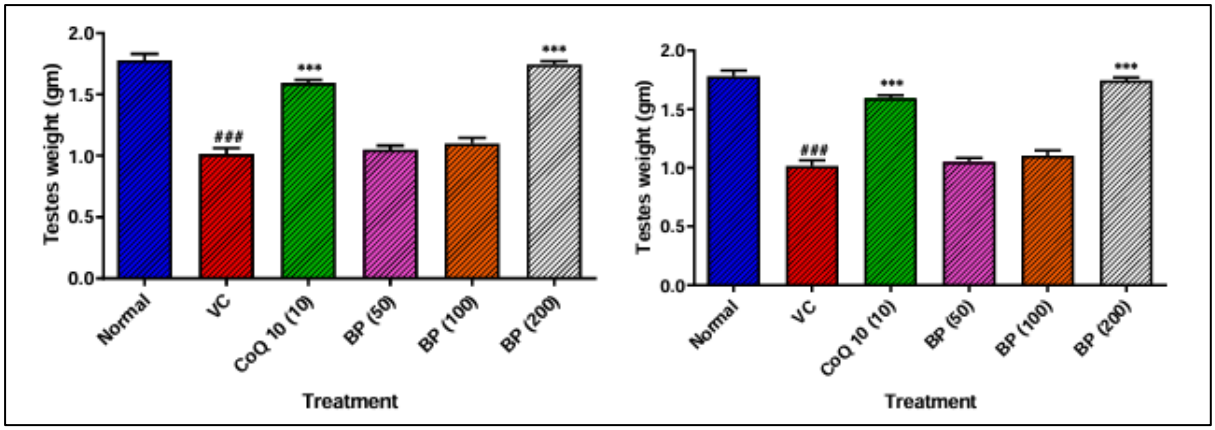

Figure 2: Effect of black pepper on arsenic-induced alteration in absolute and relative testes weights

*Data were analyzed by One-Way ANOVA followed by Dunnett's. ###P < 0.001 as compared with normal group and ***P < 0.001 as compared with Vehicle Control group on respective days.*

Effect of Standardized Black pepper Extract on Epididymal Weight

A significant reduction in absolute and relative epididymal weights was observed following sodium arsenite exposure. Absolute epididymal weight declined from 0.50 ± 0.02 g in normal animals to 0.20 ± 0.01 g in the vehicle-control group ($P < 0.001$), while relative epididymal weight decreased from $1.79 \pm 0.07 \times 10^{-3}$ to $0.91 \pm 0.07 \times 10^{-3}$. CoQ10 significantly restored both parameters. Similarly, administration of standardized black pepper extract at 200 mg/kg increased absolute epididymal weight to 0.47 ± 0.03 g and relative epididymal weight to $1.80 \pm 0.09 \times 10^{-3}$, both significantly higher than the vehicle-control group ($P < 0.001$). No statistically significant improvement was observed with the 50 or 100 mg/kg doses.

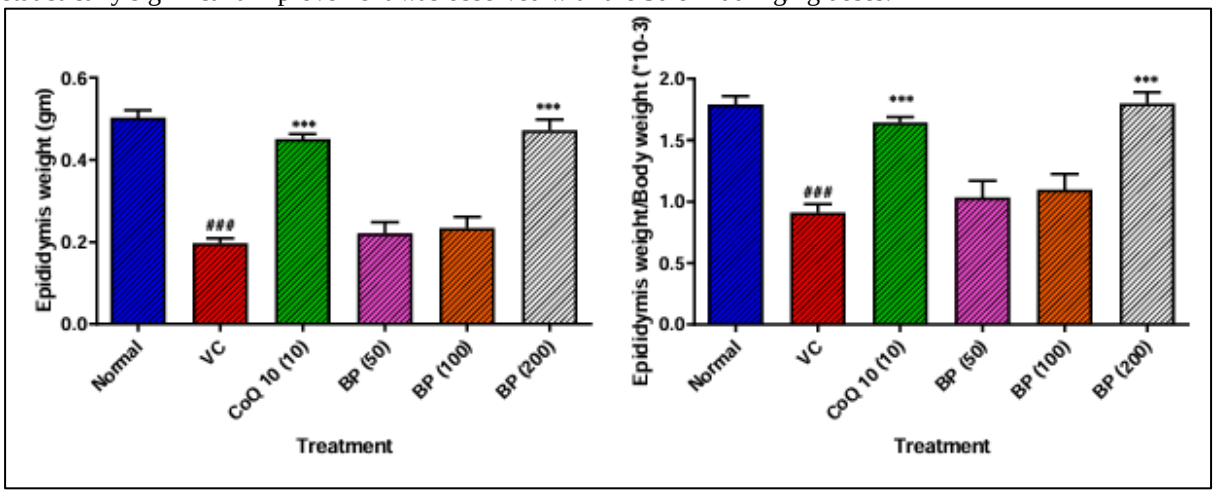

Figure 3: Effect of black pepper on arsenic-induced in absolute and relative epididymis weights

*Data were analyzed by One-way ANOVA followed by Dunnett's test. ###P < 0.001 as compared with normal group and ***P < 0.001 as compared with Vehicle Control group.*

Effect of Standardized Black pepper Extract on Prostate Weight

Sodium arsenite treatment significantly decreased both absolute and relative prostate weights. Absolute prostate weight was reduced from 608.70 ± 10.13 mg in normal rats to 310.20 ± 8.24 mg in vehicle-control animals ($P < 0.001$), while relative prostate weight decreased from 2.16 ± 0.03 to 1.43 ± 0.05 mg/g. CoQ10 treatment significantly attenuated these changes. The 200 mg/kg black pepper extract group showed a significant increase in absolute prostate weight (546.40 ± 10.98 mg) and relative prostate weight (2.09 ± 0.05 mg/g) compared with the vehicle-control group ($P < 0.001$). The lower doses did not produce statistically significant improvement.

Effect of Standardized Black pepper Extract on Sperm Count and Motility

Sodium arsenite exposure substantially impaired sperm parameters. Sperm count decreased from 59.67 ± 1.20 million/mL in the normal group to 33.50 ± 0.92 million/mL in the vehicle-control group ($P < 0.001$). Sperm motility was similarly reduced from 71.37 ± 1.04% to 39.84 ± 1.07% ($P < 0.001$). CoQ10 significantly improved both sperm count and motility. Among the black pepper-treated animals, the 200 mg/kg dose significantly increased sperm count to 48.67 ± 1.26 million/mL and sperm motility to 59.90 ± 0.65% compared with the vehicle-control group ($P < 0.001$). The 50 and 100 mg/kg doses did not result in statistically significant changes.

Effect of Standardized Black pepper Extract on Dead and Abnormal Sperm

The vehicle-control group demonstrated a significant increase in dead and morphologically abnormal sperm

following sodium arsenite exposure. The proportion of dead sperm increased from 28.45 ± 1.09% in normal animals to 59.78 ± 1.04% in the vehicle-control group, while abnormal sperm increased from 11.89 ± 0.52% to 36.32 ± 0.42% ($P < 0.001$ for both parameters). CoQ10 significantly reduced both abnormalities. The 200 mg/kg black pepper extract treatment reduced dead sperm to 41.25 ± 0.67% and abnormal sperm to 19.85 ± 0.72%, representing significant improvements compared with vehicle control ($P < 0.001$). The lower black pepper doses did not produce significant changes.

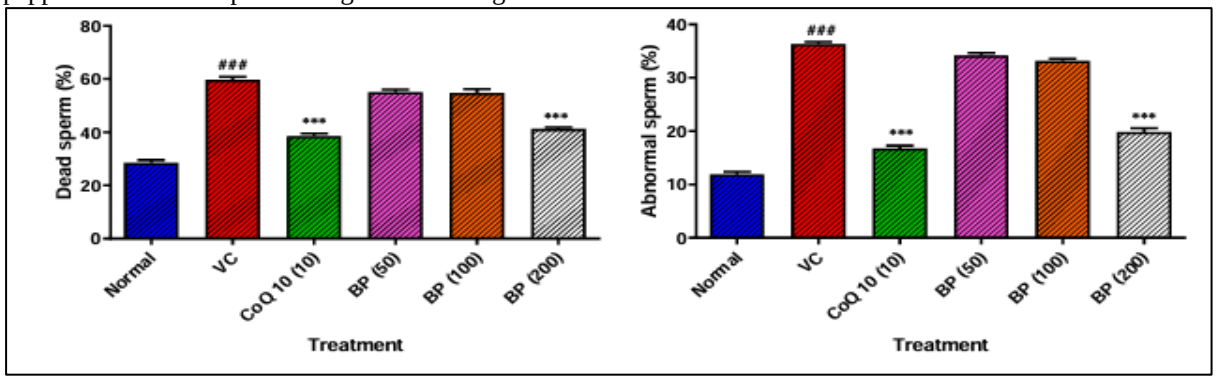

Figure 4: Effect of black pepper on arsenic-induced alteration in dead sperm and abnormal sperm

*Data were analyzed by One-way ANOVA followed by Dunnett's test. ###P < 0.001 as compared with normal group and ***P < 0.001 as compared with Vehicle Control group.*

Effect of Standardized Black pepper Extract on Serum Testosterone

Sodium arsenite administration caused a pronounced reduction in serum testosterone concentration. Testosterone levels decreased from 3.79 ± 0.08 ng/mL in normal animals to 1.24 ± 0.08 ng/mL in the vehicle-control group ($P < 0.001$). CoQ10 treatment did not produce a statistically significant increase in testosterone compared with the vehicle-control group. In contrast, administration of standardized black pepper extract at 200 mg/kg significantly increased serum testosterone to 2.86 ± 0.09 ng/mL compared with vehicle control ($P < 0.001$). The 50 and 100 mg/kg doses did not significantly restore testosterone concentrations.

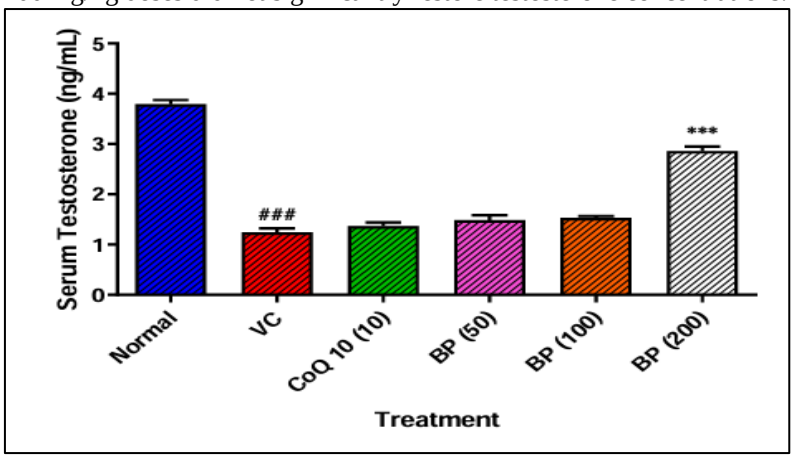

Figure 5: Effect of black pepper on arsenic-induced alteration in serum testosterone level

*Data were analyzed by One-way ANOVA followed by Dunnett's test. ###P < 0.001 as compared with normal group and ***P < 0.001 as compared with Vehicle Control group.*

Effect of Standardized Black pepper Extract on Testicular Total Protein

Testicular total protein was significantly reduced following sodium arsenite administration, declining from 10.56 ± 0.77 mg/g in the normal group to 4.99 ± 0.72 mg/g in vehicle-control animals ($P < 0.001$). CoQ10 significantly increased testicular protein concentration to 10.75 ± 0.82 mg/g compared with vehicle control ($P < 0.001$). Black pepper extract also produced a dose-related improvement. The 100 mg/kg dose increased protein concentration to 7.39 ± 0.78 mg/g ($P < 0.05$), while the 200 mg/kg dose increased it to 7.85 ± 0.94 mg/g ($P < 0.01$) compared with vehicle control.

Effect of Standardized Black pepper Extract on Testicular SOD and GSH

Sodium arsenite exposure significantly depleted endogenous antioxidant defenses. Testicular SOD activity decreased from 19.84 ± 1.65 U/mg protein in normal animals to 9.06 ± 1.33 U/mg protein in the vehicle-control group ($P < 0.001$). Similarly, GSH concentration decreased from 5.21 ± 0.39 to 1.05 ± 0.23 µg/mg protein ($P < 0.001$). CoQ10 significantly restored both antioxidant parameters. The 200 mg/kg black pepper extract treatment significantly increased SOD activity to 17.95 ± 1.70 U/mg protein and GSH concentration to 3.76 ± 0.58 µg/mg protein compared with vehicle control ($P < 0.001$ for both).

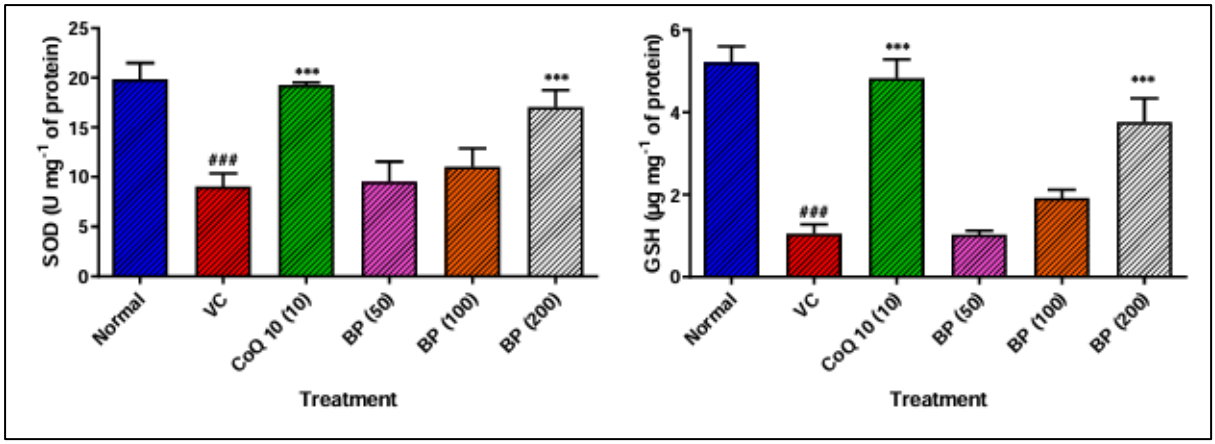

Figure 6: Effect of black pepper on arsenic-induced alteration in testicular SOD and GSH level *Data were analyzed by One-way ANOVA followed by Dunnett's test. ###P < 0.001 as compared with normal group and ***P < 0.001 as compared with Vehicle Control group.*

Effect of Standardized Black pepper Extract on Testicular MDA and Nitric Oxide

Sodium arsenite markedly increased markers associated with oxidative and nitrosative stress. Testicular MDA increased from 13.45 ± 2.85 nM/mg protein in normal rats to 49.19 ± 2.16 nM/mg protein in the vehicle-control group ($P < 0.001$). Nitric oxide also increased from 99.62 ± 5.54 to 242.30 ± 9.27 µmol/mg ($P < 0.001$). CoQ10 significantly reduced both parameters. Treatment with black pepper extract at 200 mg/kg decreased MDA to 26.86 ± 3.12 nM/mg protein and nitric oxide to 141.50 ± 7.03 µmol/mg, with both reductions being significant compared with vehicle control ($P < 0.001$).

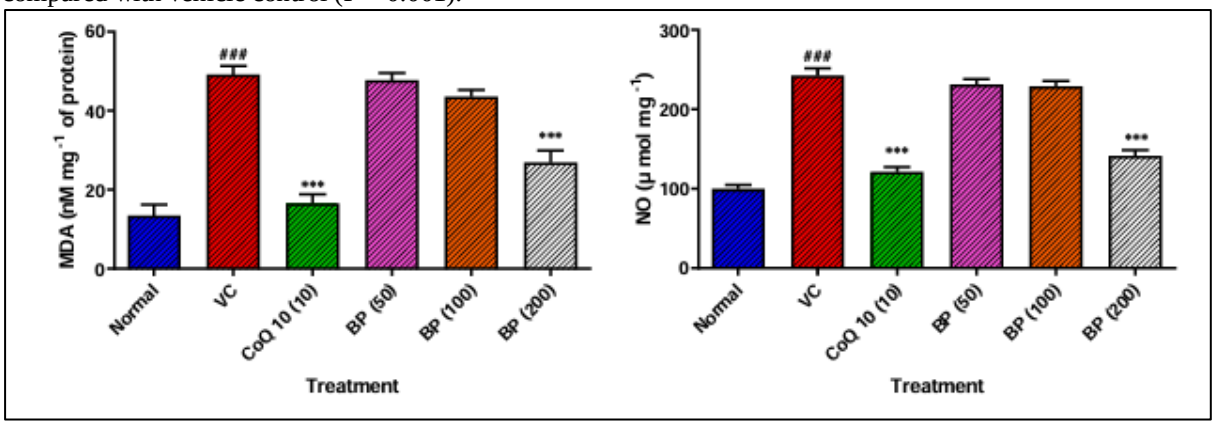

Figure 7: Effect of black pepper on arsenic-induced alteration in testicular MDA and NO level
*Data were analyzed by One-way ANOVA followed by Dunnett's test. ###P < 0.001 as compared with normal group and ***P < 0.001 as compared with Vehicle Control group.*

Table 1: effect of standardized *Piper nigrum* extract on sodium arsenite-induced reproductive toxicity in rats

Parameter	Normal	Sodium arsenite control	CoQ10 (10 mg/kg)	BP (50 mg/kg)	BP (100 mg/kg)	BP (200 mg/kg)
Body weight (g)	281.30 ± 2.77	217.50 ± 3.82###	274.30 ± 2.72	214.70 ± 2.79	212.20 ± 4.01	262.00 ± 2.72***
Absolute testes weight (g)	1.78 ± 0.05	1.01 ± 0.05###	1.60 ± 0.03	1.05 ± 0.03	1.10 ± 0.05	1.74 ± 0.03***
Relative testes weight (×10⁻³)	6.33 ± 0.21	4.66 ± 0.23###	5.82 ± 0.11	4.90 ± 0.21	5.19 ± 0.18	6.66 ± 0.17***
Absolute epididymis weight (g)	0.50 ± 0.02	0.20 ± 0.01###	0.45 ± 0.01	0.22 ± 0.03	0.23 ± 0.03	0.47 ± 0.03***
Relative epididymis weight (×10⁻³)	1.79 ± 0.07	0.91 ± 0.07###	1.64 ± 0.05	1.04 ± 0.14	1.10 ± 0.13	1.80 ± 0.09***
Absolute prostate weight (mg)	608.70 ± 10.13	310.20 ± 8.24###	560.10 ± 10.80	296.80 ± 10.71	294.60 ± 10.28	546.40 ± 10.98***
Relative prostate weight (mg/g)	2.16 ± 0.03	1.43 ± 0.05###	2.04 ± 0.05	1.38 ± 0.05	1.39 ± 0.07	2.09 ± 0.05***

Sperm count (million/mL)	59.67 ± 1.20	33.50 ± 0.92###	57.17 ± 1.33	33.50 ± 0.72	34.83 ± 1.20	48.67 ± 1.26***
Sperm motility (%)	71.37 ± 1.04	39.84 ± 1.07###	62.03 ± 1.03	44.59 ± 0.90	43.48 ± 1.51	59.90 ± 0.65***
Dead sperm (%)	28.45 ± 1.09	59.78 ± 1.04###	38.51 ± 1.02	55.02 ± 1.01	54.77 ± 1.44	41.25 ± 0.67***
Abnormal sperm (%)	11.89 ± 0.52	36.32 ± 0.42###	16.75 ± 0.53	34.18 ± 0.49	33.15 ± 0.44	19.85 ± 0.72***
Serum testosterone (ng/mL)	3.79 ± 0.08	1.24 ± 0.08###	1.37 ± 0.07	1.49 ± 0.10	1.53 ± 0.04	2.86 ± 0.09***
Testicular total protein (mg/g)	10.56 ± 0.77	4.99 ± 0.72###	10.75 ± 0.82	5.38 ± 1.03	7.39 ± 0.78*	7.85 ± 0.94**
Testicular SOD (U/mg protein)	19.84 ± 1.65	9.06 ± 1.33###	19.27 ± 0.25	9.53 ± 2.02	11.01 ± 1.90	17.95 ± 1.70***
Testicular GSH (µg/mg protein)	5.21 ± 0.39	1.05 ± 0.23###	4.82 ± 0.47	1.02 ± 0.11	1.91 ± 0.20	3.76 ± 0.58***
Testicular MDA (nM/mg protein)	13.45 ± 2.85	49.19 ± 2.16###	16.57 ± 2.35	47.97 ± 1.88	43.52 ± 1.82	26.86 ± 3.12***
Testicular NO (µmol/mg)	99.62 ± 5.54	242.30 ± 9.27###	121.40 ± 6.05	231.20 ± 7.22	299.10 ± 6.85	141.50 ± 7.03***

Histopathological Effect of Standardized Black pepper Extract on Testicular Tissue

Histopathological examination revealed marked differences among the experimental groups. Testicular sections from normal animals showed largely preserved tissue architecture, with minimal inflammatory infiltration and no evident necrosis or Leydig cell damage. In contrast, sodium arsenite-treated vehicle-control animals exhibited extensive pathological alterations, including severe inflammatory infiltration, necrosis, Leydig cell damage, and vacuolation of spermatogenic cells. CoQ10 treatment markedly reduced these pathological changes, with inflammatory infiltration and necrosis reduced to grade 2 and Leydig cell damage and spermatogenic-cell vacuolation reduced to grade 1. Treatment with standardized black pepper extract at 200 mg/kg also improved testicular morphology, with inflammatory infiltration, necrosis, and Leydig cell damage graded as 2 and vacuolated spermatogenic cells graded as 1. These findings indicate partial restoration of testicular tissue architecture following high-dose black pepper treatment.

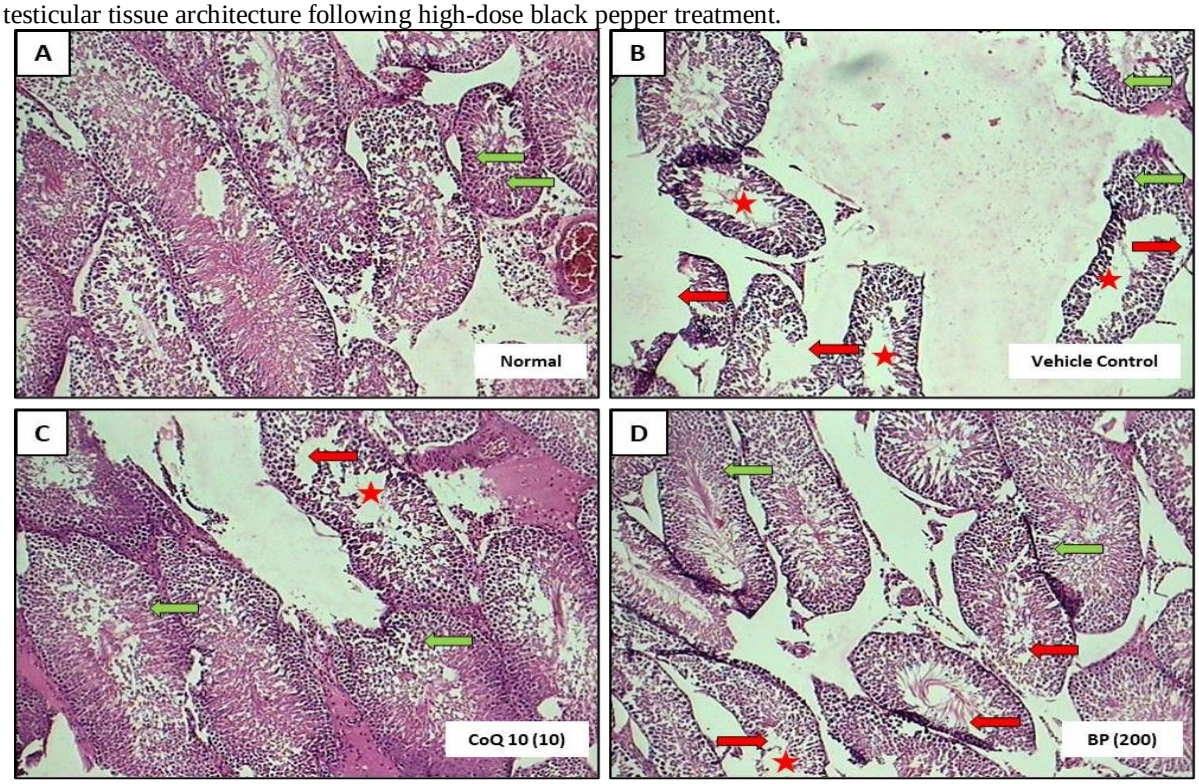

Figure 8: Histopathological representation of testicular tissue from normal rats (A), Vehicle Control rats (B), CoQ 10 (10 mg/kg) treated rats (C), BP (200 mg/kg) treated rats (D). Stained with H&E (at 100 X).

DISCUSSION:

The present study demonstrated that sodium arsenite produced substantial reproductive toxicity in rats, as evidenced by reductions in body weight, reproductive-organ weights, sperm count, sperm motility, and serum testosterone, together with marked oxidative stress and testicular histopathological alterations. These findings indicate considerable impairment of male reproductive function following arsenite exposure.

A pronounced disturbance in testicular redox status was observed after sodium arsenite administration. Testicular SOD and GSH were markedly reduced, whereas MDA and NO levels were elevated, indicating increased oxidative and nitrosative stress. Treatment with standardized *Piper nigrum* extract at 200 mg/kg significantly improved SOD and GSH and reduced MDA and NO compared with the arsenite control group. These findings suggest that improvement of endogenous antioxidant defenses may contribute to the observed protective effect of the extract.

Sodium arsenite also adversely affected reproductive performance, as demonstrated by significant decreases in sperm count and motility and increases in dead and abnormal sperm. The 200 mg/kg dose of standardized black pepper extract significantly improved sperm count and motility while reducing dead and abnormal sperm compared with the arsenite control. This improvement was accompanied by recovery of reproductive-organ weights, particularly testicular, epididymal, and prostate weights. The hormonal findings further supported the protective activity of the extract. Sodium arsenite markedly reduced serum testosterone, whereas the 200 mg/kg black pepper treatment significantly increased testosterone compared with the arsenite control. The extract also improved testicular total protein levels, suggesting attenuation of arsenite-associated tissue injury. Histopathological examination provided additional evidence of testicular protection. Sodium arsenite produced extensive inflammatory infiltration, necrosis, Leydig cell damage, and vacuolation of spermatogenic cells. Administration of black pepper extract at 200 mg/kg reduced these pathological alterations and was associated with improved preservation of testicular architecture.

Overall, the findings suggest that the protective activity of standardized P. nigrum extract is associated primarily with attenuation of oxidative/nitrosative stress and preservation of reproductive tissue and function. The present data support the involvement of antioxidant and cytoprotective effects; however, specific anti-inflammatory, anti-apoptotic, or molecular mechanisms were not directly measured in this study and therefore should not be presented as established mechanisms.

CONCLUSION:

The present study demonstrated that sodium arsenite induced significant reproductive toxicity in rats, characterized by impaired reproductive-organ parameters, deterioration of sperm quality, reduced testosterone and testicular protein, depletion of SOD and GSH, increased MDA and NO, and marked histopathological damage. Standardized *Piper nigrum* extract, particularly at 200 mg/kg, significantly attenuated several of these alterations and improved reproductive, biochemical, and histological parameters. These findings indicate that standardized black pepper extract has potential protective activity against sodium arsenite-associated reproductive toxicity, with the observed effects being consistent with improvement of oxidative stress and preservation of testicular function. Further studies investigating molecular mechanisms, dose optimization, long-term safety, and translational relevance are warranted before considering potential clinical application.

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All authors have contributed equally.

CONFLICT OF INTERESTS:
All authors have declared that there is no conflict of interest.

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