

*Regular Research Paper*

# Ameliorative prowess of quercetin on the cytoarchitecture of testes in Sprague-Dawley rats exposed to formaldehyde

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**Formaldehyde is a metabolic product of methanol that is further broken down into formic acid. Long-term exposure to formaldehyde has been reported to cause adverse health effects. This study was designed to investigate whether quercetin could mitigate formaldehyde-induced damage to the reproductive organs. Forty rats were used and divided into four groups. Group A served as the control and was not exposed to formaldehyde, while Groups B–D served as treatment groups and were exposed to 40% formaldehyde for 1, 2, and 3 h, respectively. At the end of the exposure period, five animals from each group were randomly selected and euthanized. The remaining five animals in each group received quercetin at a dose of 100 mg/kg for four weeks to evaluate its potential ameliorative effect, after which they were euthanized. The results showed a reduction in body weight and hormonal parameters in the treatment groups compared with the control group. Histological examination revealed distorted seminiferous tubules and absence of spermatozoa in the lumen of animals exposed to formaldehyde, including those treated with quercetin. In conclusion, both hormonal and histological analyses demonstrated that formaldehyde exerted adverse effects on the histoarchitecture of the testes, and quercetin was unable to ameliorate the toxicity induced by formaldehyde.**

**Key words:** Formaldehyde, testes, hormonal milieu, Sprague-Dawley rats, Quercetin.

## INTRODUCTION

Formaldehyde is basically defined as a colorless and unstable gas with a suffocating odor. It has a simple chemical formula of CH<sub>2</sub>O. It is a product of methanol that further breaks down into formic acid (Haidry et al., 2023). Given its small size and light nature, formaldehyde readily

evaporates from the surface of formalin, a solution of formaldehyde in water. The pace at which this happens is dependent upon temperature, humidity, and air flow (Robert and Nallanthambi, 2021). Van der Waals force and other intermolecular barriers are overcome more readily by

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formaldehyde molecules as they move from the liquid phase into the gas phase in thinner liquid layers where the formaldehyde is dissolved (Suen et al., 2022). It is also known to contain reactive molecules that have cytotoxic effect (Bernardini et al., 2022).

Extended exposure to high concentrations of formaldehyde, particularly in work environments, has been identified as the source of discomfort and discomfort, including upper and lower respiratory tract irritation, ocular irritation, degenerative illnesses, wheezing, coughing, body wounds, pain in the chest, discomfort in the stomach, and appetite loss (Kim et al., 2011). Chronic and serious health effects, including inflammation and hyperplastic alters of the nasal mucous membrane, pharyngeal congestion, persistent pharyngitis, chronic rhinitis, loss of olfactory functioning, lacrimation and corneal disorder, heartburn, tremor, and lethargy, have also been linked to long-term occupational formaldehyde exposure (Feron et al., 2001). Song et al. (2015) stated that formaldehyde can change micro-RNA patterns linked to the control of gene expression, which may cause a number of diseases to start. In a pathology investigation carried out by Murta et al. (2016), increased levels of malondialdehyde-deoxyguanosine adduct, a biomarker of oxidative stress and lipid peroxidation, in leukocytes were discovered to indicate that exposure to formaldehyde levels above  $66 \mu\text{g m}^{-3}$  may cause oxidative stress.

Studies have reported the harmful effect of formaldehyde on exposure to testes and ovaries in experimental models. Wang et al. (2013) reported on the histopathological findings that vascular congestion and interstitial edema in the ovaries, as well as a considerable decrease in the quantity and size of mature follicles. A report by Naghdi et al. (2016) showed that the structure of these vacuoles indicates the death or exfoliation of clones of spermatogenic cell lines. The seminiferous epithelium was disordered, and the epithelium of the seminiferous tubules looked heavily vacuolated. There were also some seminiferous tubules that showed signs of spermatogenic cycle arrest. In the lumen, detached germ cells or deteriorating germinal components were present.

Quercetin belongs to the group of plant pigments called flavonoids. It can be found as the conjugate of a glucose or a glycone. There are five distinct locations for the five hydroxyl groups in this 3, 5, 7, 3, 4-pentahydroxyflavon. The following edibles include its derivatives in varying amounts: buckwheat, chokeberry, mango, chokeberry, chokeberry, beans, lettuce, onions, cranberries, blueberries, and chokeberries (Singh et al., 2004; Loke et al., 2008; Zahedi et al., 2013). It tastes harsh and is a crystalline solid. This is a class of naturally occurring flavonoids that are connected by a heterocyclic pyrone ring to the nucleus of common flavone that consists of more than one ring on the benzene

structure. Since humans and other animals are unable to synthesize the flavone nucleus; only the kingdom Plantae is capable of producing flavonoids (Baghel et al., 2012). Quercetin is a strong antioxidant because it may scavenge free radicals and bind transition metal ions.

According to Sakanashi et al. (2008), quercetin reduces lipid peroxidation through its antioxidant mechanism. Lipid peroxidation has been shown to exert harmful effects on the human body, as it damages cellular membranes and biomolecules. Antioxidants such as quercetin can inhibit this process by interacting with and neutralizing free radicals. In addition, quercetin scavenges free radicals and reduces inflammation in affected tissues. Free radicals are known to activate transcription factors that stimulate the production of pro-inflammatory cytokines (D'Andrea, 2015).

Quercetin has also been reported to protect tissues against reperfusion-induced ischemic damage by scavenging free radicals (Baghel et al., 2012). It prevents tissue injury through multiple mechanisms, including the direct neutralization of reactive oxygen species. Quercetin, a flavonoid, has been shown to reduce low-density lipoprotein (LDL) oxidation in vitro by scavenging free radicals, thereby inhibiting the development of atherosclerosis (Kerry and Abbey, 1997).

A study by Adarmanabadi et al. (2023) reported that quercetin has beneficial effects on metabolic disorders, male infertility, and cancer. Oxidative stress and inflammation play critical roles in the pathophysiology of the male reproductive system and contribute significantly to infertility. Owing to its antioxidant and anti-inflammatory properties, quercetin may serve as an adjuvant therapeutic agent for male infertility (Oyovwi et al., 2023). Numerous studies have investigated the effects of quercetin on male fertility and infertility. Evidence indicates that quercetin can chelate toxic heavy metals and function as a potent antioxidant and anti-inflammatory compound (Azeem et al., 2023).

Both beneficial and adverse effects of quercetin and other polyphenols on male fertility have been documented (Almujaydil et al., 2023; Martin and Touaibia, 2024). However, the majority of studies highlight quercetin's antioxidative properties and its role in the prevention and treatment of male infertility (Mohlala et al., 2023; Chavda et al., 2024).

Quercetin has several notable benefits, including the reduction of hydrogen peroxide production, enhancement of sperm antioxidant capacity, and prevention of oxidative stress-induced DNA damage (Moretti et al., 2012). It has also been shown to attenuate oxidative damage by increasing the activities of antioxidant enzymes such as glutathione peroxidase and superoxide dismutase, particularly in diethylstilbestrol (DES)-induced oxidative stress models. Numerous studies have further reported the

protective effects of quercetin against oxidative stress in human spermatozoa (Moretti et al., 2012).

The study aims at using quercetin as a potential ameliorative agent to mitigate the deleterious effects of formaldehyde on testes. A special kind of bioflavonoid known as quercetin can be found in a wide range of plant foods, including apples, berries, Brassica vegetables, capers, grapes, onions, shallots, tea, and tomatoes. It can also be found in many seeds, nuts, flowers, barks, and leaves, where it is present as a glycoside (Adebajo et al., 2022). The most aptly recognized characteristic of quercetin is its antioxidant activity. When it comes to defending the body against reactive oxygen species, which are either created naturally during oxygen metabolism or brought on by external damage, quercetin appears to be the most potent flavonoid (Shah et al., 2016), hence, its choice as for this study.

## MATERIALS AND METHODS

### ETHICAL APPROVAL

The College of Health Science at Bowen University, Iwo Campus, Osun State, granted ethical permission through the Research and Ethical Committee, with a reference number BUI/COHES/ANA/012/033

### CARE AND MANAGEMENT OF ANIMALS.

A total of 40 adult of about 6-8 weeks old Sprague-Dawley rats with weights within the range of  $150 \pm 50$  g were used for the experiment. The animals were bought from Peter's Farms in Badagry, Lagos State, and housed in the Anatomy Program's Animal House Center at Bowen University's Iwo campus in Osun State. After two weeks of acclimatization, the animals were given unlimited access to standard rat chaw and water when the benchwork began, and they were kept there for 28 days.

### ANIMAL GROUPING AND ADMINISTRATION

The rats were randomly selected and put in 4 groups of 10 rats in each group designated as A-D. The route of administration was via inhalation of the 40% formaldehyde in which the animals were placed in a cage specially designed for the fume cupboard. The fume cupboard had two compartments, one above and one below with perforations made on the platform. The rats were placed in the superior compartment while the 750 ml of 40% formaldehyde was poured into a container below allowing them to only inhale the gas not coming in contact with the chemical solution. The pure quercetin used in this study was obtained from Fairy Pharmaceuticals Ltd., Zhuhai City, Guangdong Province, China. The product had a manufacturing date of June 2022 and an expiry date of May 2027.

Group A serves as control and the animals were not exposed to formaldehyde, Groups B-D serve as treatment groups and the animals were exposed to 40% formaldehyde for a period of 1, 2 and 3 h, respectively. Upon completion of this benchwork, 5 animals from groups A-D were randomly selected and euthanized. The remaining

five animals in each group received quercetin at a dose of 100 mg/kg starting on day 29 of the study to assess its potential ameliorative effect. Treatment continued for 28 days, after which the animals were euthanized.

### ANIMAL SACRIFICE

Upon the completion of the bench work, the respective animals were euthanized with 1 ml of Ketamine. Immediately after, fresh blood for hormonal analysis was obtained via cardiac puncture, after which their reproductive organs were harvested via abdomino-pelvic dissection, weighed accurately using the sensitive weighing balance and immediately fixed in specimen bottles containing Bouin's fluid. The blood collected was stored in the EDTA bottle and centrifuged on the same day. The supernatant was then separated and placed in the freezer at  $-80^{\circ}\text{C}$  before it was transported to the laboratory for analysis as described by Adebajo et al. (2022).

### CHEMICALS

Formaldehyde was purchased from Agridirect, Drumrahan retail, Ltd, Ireland and quercetin was acquired from St. Louis, Missouri's Sigma Chemical Company.

### STATISTICS

Using Graph Pad software version 9.5, ONE WAY-ANOVA was used to gather and statistically evaluate the data collected from each group. The data results were presented as mean  $\pm$  SEM (standard error of mean), with a significant threshold of  $p < 0.05$ .

## RESULTS

### Impact of oral quercetin administration and formaldehyde exposure on adult SPRAGUE-DAWLEY rats' body weight

In the values of the body weight, significant increase was recorded in both formaldehyde and quercetin groups when after exposure was compared to before exposure (Table 1)

### Impact of oral quercetin administration and formaldehyde exposure on adult Sprague-Dawley rats' hormonal milieu

In the formaldehyde-exposed groups, comparison of the treatment groups (B-D) with the control group (A) revealed a significant, dose-dependent increase in hormonal parameters. However, a reduction in these values was observed when the quercetin-treated groups were compared with the formaldehyde-only groups, with statistically significant differences noted in Groups C and D (Table 2).

**Table 1.** Impact of oral quercetin administration and formaldehyde exposure on adult Sprague-Dawley rats' body weight.

Formaldehyde			
Group (Duration of exposure)	Before exposure	After exposure	% body weight difference
A (0 h)	126.33 ± 2.11	145.72 ± 1.18*	13.31
B (1 h)	131.04 ± 1.30	120.02 ± 0.45*	9.18
C (2 h)	140.95 ± 0.95	129.94 ± 2.49*	8.47
D (3 h)	166.04 ± 2.01	158.84 ± 3.01*	4.53
Quercetin			
Group (Duration of exposure)	Before exposure	After exposure	% body weight difference
A (0 h)	124.81 ± 1.63	145.93 ± 0.94	14.47
B (1 h)	144.92 ± 2.02	153.09 ± 3.44	5.34
C (2 h)	160.43 ± 2.61	170.11 ± 2.26	5.69
D (3 h)	183.10 ± 0.76	190.83 ± 1.06	4.05

Values are mean standard error of mean; n=5, \* <0.05 (student's T test).

**Table 2.** impact of oral quercetin administration and formaldehyde exposure on adult Sprague-Dawley rats' hormonal milieu.

Formaldehyde			
Group	FSH	LH	Testosterone
A (0 h)	0.83 ± 0.20	1.61 ± 0.01	6.22 ± 1.33
B (1 h)	1.47 ± 0.14 <sup>a</sup>	2.19 ± 0.83 <sup>a</sup>	7.49 ± 0.28 <sup>a</sup>
C (2 h)	1.71 ± 0.15 <sup>a</sup>	4.55 ± 1.73 <sup>ab</sup>	9.65 ± 0.86 <sup>ab</sup>
D (3 h)	3.33 ± 2.14 <sup>abc</sup>	6.01 ± 0.14 <sup>abc</sup>	10.44 ± 0.06 <sup>abc</sup>
Quercetin			
Group	FSH	LH	Testosterone
A (0 h)	0.92 ± 1.01	1.76 ± 0.02	6.48 ± 1.04
B (1 h)	1.20 ± 0.02	1.96 ± 0.02	7.01 ± 0.17
C (2 h)	1.42 ± 0.11 <sup>a*</sup>	2.85 ± 0.01 <sup>ab*</sup>	8.94 ± 0.40 <sup>ab*</sup>
D (3 h)	2.03 ± 0.02 <sup>abc*</sup>	3.06 ± 0.03 <sup>abc*</sup>	9.03 ± 0.03 <sup>ab*</sup>

LH is luteinizing hormone; FSH is follicle stimulating hormone.

Values are expressed as mean ± standard error of the mean (SEM). <sup>a</sup>p < 0.05 indicates a significant difference compared with the control group; <sup>b</sup>p < 0.05 indicates significance compared with the low-dose group; <sup>c</sup>p < 0.05 indicates significance compared with the medium-dose group; and \*p < 0.05 indicates significance compared with the quercetin-treated group.

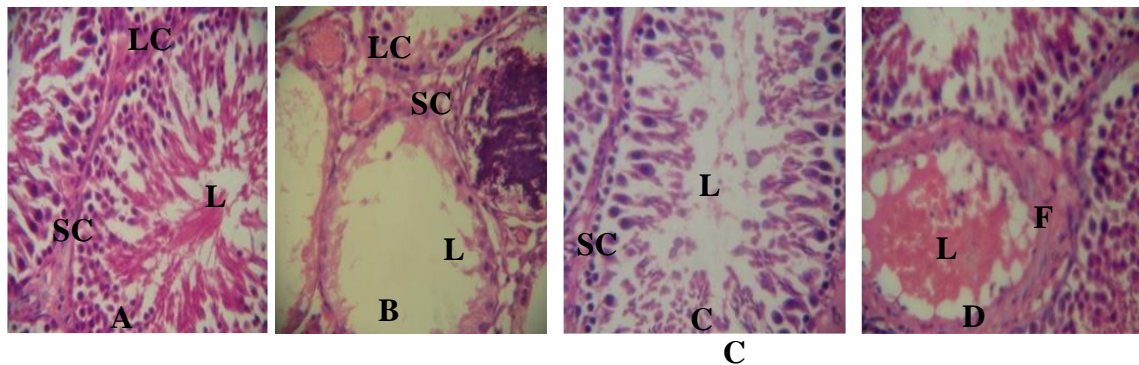
#### Effect of exposure to formalin on the cyto-architecture of testes in adult Sprague-Dawley rats (H and E stain; Magnification X 400)

Figure 1 (A) demonstrates normal spermatozoa threads within the lumen (L), with intact Sertoli cells (SC) and a normal developing germinal cell layer. The interstitial (Leydig) cells (LC) also appear normal. Figure 1(B) shows complete depletion of Sertoli cells (SC), with a few

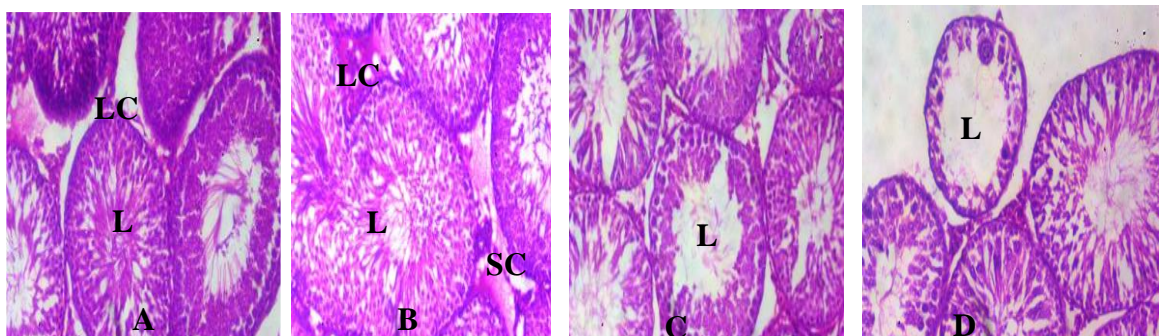
spermatogonial cells evident in some seminiferous tubules (L). Several tubules exhibit maturation arrest, while the interstitial (Leydig) cells appear normal. Figure 1(C) reveals a reduced population of Sertoli cells (SC). The lumen (L) contains residual cells and a few strands of spermatozoa, whereas the interstitial (Leydig) cells remain normal. Figure 1(D) shows the presence of a markedly dilated blood vessel with a thickened wall (F), along with periferal fatty infiltration and congestion within the lumen (L).

#### Effect of exposure to formalin and oral administration of quercetin on the cyto-architecture of testes in adult Sprague-dawley rats (H and E stain; Magnification X 400)

Figure 2(A) displays healthy seminiferous tubules with fully developed germinal cells, a spermatozoa-filled lumen (L),



**Figure 1.** Testicular Histology Showing (A) Normal Seminiferous Tubules with Intact Sertoli and Leydig Cells, (B) Complete Sertoli Cell Depletion with Maturation Arrest, (C) Reduced Sertoli Cell Population with Residual Lumen Cells and Few Spermatozoa, and (D) Dilated Blood Vessel with Fatty Infiltration and Luminal Congestion.



**Figure 2.** Histological Examination of Seminiferous Tubules Showing (A–B) Healthy Tubules with Fully Developed Germinal Cells, Spermatozoa-Filled Lumen, and Normal Leydig Cells, (C) Tubules with Limited Maturation Arrest, and (D) Atrophic Tubules Exhibiting Maturation Arrest and Degenerated Germ Cells.

and normal interstitial spaces containing typical Leydig cells (LC). Figure 2 (B) illustrates healthy seminiferous tubules with fully developed germinal cells, a spermatozoa-filled lumen (L), and normal interstitial spaces with typical Leydig cells (LC). Figure 2 (C) shows a small number of seminiferous tubules exhibiting maturation arrest within the lumen (L). Figure 2 (D) reveals a few atrophic seminiferous tubules with maturation arrest in the lumen (L) and evidence of degenerated germ cells.

**DISCUSSION**

Formaldehyde has been widely used in industries and medicine as disinfectants and fixative over the years. However, it is said to be an environmental contaminant. It contains reactive molecules which have been known to have

the ability to react with tissue proteins and have cytotoxic effect (Eastmond et al., 2014). There has also been reports of menstrual irregularities in female workers exposed to formaldehyde (Khoshakhlagh et al., 2024). In contrast to work carried out by Razi et al. (2013). In comparison to the control group, which experienced a placebo effect from ambient oxygen, this study demonstrates elevated serum testosterone levels in the experimental groups. This increase was seen to follow a trend with the control having the lowest value and group D (3 h exposure) having the highest value.

Additionally, a statistically significant difference was seen between the formaldehyde-exposed group and the control group in the study of the serum levels of FSH and LH. This demonstrates that the formaldehyde exposure was the cause of the value discrepancy. Testicular histology revealed that the experimental groups' cytoarchitecture was

distorted in comparison to the control group. These changes included a decrease in mature spermatocytes, vacuolation, vascular congestion, and Sertoli cell depletion. In other words, all these alterations can be said to be due to the effect of exposure to formaldehyde. Quercetin is one of the major flavonoids and it is widely distributed in edible plants such as onions, potatoes and apples and one of the most potent antioxidants of plant origin. It has been shown to have highly potent antioxidant and cyto-protective effects in preventing apoptosis caused by oxidants (Adebajo et al., 2022). It has also been widely documented to attenuate induced reproductive damages (Zhang et al., 2023). The administration of quercetin did not improve the hormonal parameters checked as there was still dose dependent increase in the values recorded. The testicular sections showed atrophic seminiferous tubules with degenerated germ cells and maturation arrest. This could possibly affect the chances of fertility, resulting in infecundity among individuals exposed to formaldehyde.

## CONCLUSION

The results obtained from both hormonal analysis and histological studies show to a great extent that formaldehyde has an adverse effect on the histoarchitecture of the testes and quercetin could not ameliorate the toxicity caused by formaldehyde. We recommended that occupational settings control the level of formaldehyde in the air to the occupational exposure standard by adequate ventilation. If this cannot be achieved breathing protection should be used (NPIS, 2002). Additionally, measures should be implemented to reduce formaldehyde levels in gross anatomy laboratories, including the use of locally ventilated dissection worktables and the neutralization of formaldehyde with ammonium carbonate. Alternative embalming fluids containing ethyl alcohol, polyethylene glycol, or phenoxyethanol may also be used to minimize formaldehyde exposure (Frölich, 1984). Furthermore, where feasible, low-formaldehyde cross-linking agents should be adopted in textile manufacturing processes.

## CONFLICT OF INTEREST

The authors have not declared any conflict of interest

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