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Ameliorative Potentials of Quercetin in Some Histo-Physiological and Biochemical Parameters Against Alterations Induced Benzene Inhalation in Albino Rats

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Abstract

Present study investigates the protective role of quercetin in reducing benzene-induced toxicity in rats. Sixteen adult rats, weighing 200-250 g, were selected. They were divided into four groups: 1. Control group, 2. Benzene inhaled group, 3. Quercetin group and 4. Group of benzene and quercetin in combination. Biochemical, spermatological parameters, and histopathological changes in lungs were recorded. Results of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), urea and creatinine levels in the serum of rats were higher in benzene exposed rats than in unexposed rats. Conversely, exposure to benzene led to a decrease in sperm quality compared to the unexposed rats. Histopathological examinations of the lung tissues revealed structural changes in exposed rats, including emphysema, thinning of the wall of the alveolar sac, congestion between the alveolar sacs and more elasticity in the wall of smooth muscles. The present study showed that quercetin treatment can reverse the negative effects of benzene in approximately all studied parameters and showed amelioration in the histological anomalies induced by benzene toxicity.

Keywords: Benzene, Quercetin, biochemical and spermatological parameters, lung histology

القدرة التحسينية للكيرسيتين في بعض المتغيرات النسيجية الفسيولوجية والكيموحيوية ضد التغيرات التي يسببها استنشاق البنزين في الجرذان البيضاء

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الخلاصة

تبحث الدراسة الحالية الدور الوقائي للكيرسيتين في الحد من السمية الناجمة عن استنشاق بخار البنزين في الجرذان. تم تقسيم 16 جرذا إلى أربع مجموعات: المجموعة الضابطة ومجموعات المعاملة تلقت استنشاق البنزين، كيرسيتين، و مجموعة كيرسيتين و بنزين سوية. تم تقدير المعلمات الكيموحيوية والحيوانات المنوية. بالإضافة إلىالتغيرات النسيجية المرضية في الرئتين. كانت نتائج الأسبارتات أمينوترانسفيراز (AST)، ألانين أمينوترانسفيراز (ALT)، الفوسفاتيز القلوي (ALP)، اليوريا، والكرياتينين أعلى في الجرذان المعرضة منها في الجرذان غير المعرضة لاستنشاق البنزين. على العكس من ذلك، أدى التعرض للبنزين إلى انخفاض جودة

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الحيوانات المنوية مقارنة بالجرذان غير المعرضة. اظهر الفحوصات النسجية المرضية لأنسجة الرئة عن تغيرات هيكلية تركيبية في رئة الجرذان المعرضة لاستنشاق البنزين شملت انتفاخ الرئة، وترقق جدار الكيس السنخي، واحتقان بين الأكياس السنخية،مرونة أكثر في جدار العضلات الملساء في الرئة. وجدت الدراسة الحالية أن المادة المضافة كيرسيتين يمكن أن تثبط الآثار السلبية للبنزين في جميع المعلمات المدروسة تقريبا، كماأظهرت تحسنا في الحالات النسيجية الناجمة عن سمية البنزين.

1. Introduction

Benzene is a polluting volatilizable organic compound that is regularly related to the activity of mankind in the environment. These chemicals are known carcinogens that exist in our water, soil and air [1]. Environmental pollution caused by benzene originates mostly from its improper discharge from industries and automobiles exhausts. Smoking cigarettes within buildings also increases benzene levels significantly in the air [2].

Benzene is known to cause disorders in different organs and systems, including the respiratory, gastrointestinal, cardiovascular, nervous systems, kidney, liver, testis and ovary [3, 4]. The risk of serious myeloid leukemia has increased because of exposure to benzene [5]. The potential locations where people can be exposed to benzene might include driving vehicles, working or visiting the gas station, working in the automobile workshop and living close to petroleum refineries or chemical manufacturing plants. With each inspiration, the vapour of benzene is quickly absorbed into the bloodstream and circulated all through the body. Pathways for exposure to benzene in humans are pulmonary and dermal [6]. Benzene content in soil can pose a potential risk to human health and disrupt ecological systems' variety and structure [4].

The toxicity of benzene is linked with its metabolism. The metabolites involved are catechol, hydroquinone, phenol and possibly benzene oxide [7]. It is recognized as a human carcinogen. Epidemiological studies have shown that it causes acute and chronic leukemia, even at low dosages [8]. Acute benzene exposure might affect the central nervous system, causing dizziness, nausea and headaches, whereas chronic exposure can result in more serious negative health effects such as genotoxicity, haematotoxicity, reproductive effects, and elevated levels of persistent chromosome aberrations and mortality [9].

Therefore, there is an urgent need to establish strategies for neutralizing the negative effects of benzene on the histological, physiological and biochemical characteristics of different organs. Some nutritional antioxidants can protect cells from the negative effects of environmental oxidant material. Many bioflavonoids are free radical scavengers, antioxidants, and modifiers of numerous enzymatic and biological processes of all bioflavonoids. Quercetin is one of the most frequently distributed dietary polyphenolic compounds and has highest antioxidant capability [10]. Quercetin is found in numerous vegetable foods, such as apples, onion, broccoli and leafy green vegetables, which give many fruits, flowers and vegetables their colour, quercetin has several physiological properties, including anti-inflammatory and antioxidative, reduces inflammation. It may also enhance the effects of anti-cancer drugs [11]. The role of free radical scavengers includes damaging cell membrane, manipulate DNA and potentially even kill the cells [12]. However, a previous study demonstrated that quercetin was poorly absorbed from the digestive tract and did not significantly influence the organs when administrated orally [13].

The current study aims to assess the risks associated with chronic low-level exposure scenarios of benzene and evaluate the effects of quercetin against benzene inhalation in rats on different histophysio biochemical biomarkers.

2. Materials and Methods

2.1 Chemicals

Quercetin (99% purity) was procured from Beijing Solarbio Science and Technology Co., Ltd. (Beijing, China), and benzene was obtained from Sigma-Aldrich Co., USA. All other chemicals and laboratory kits were purchased from local suppliers.

2.2 Animals

Sixteen adult male albino rats of Wistar strain, weighing between 200-250 g ,were provided by the animal house in the Department of Biology, College of Education, Salahaddin University, Erbil. The rats were housed in clean polypropylene cages (four rats per cage) that were maintained in an air-conditioned animal house under temperature 22±2°C, with constant photoperiods of 12h light/dark cycle. They were fed with a pellet diet and water *ad libitum*. The animals were allowed to acclimate for seven days before treatment.

2.3 Experimental Design

The animals were divided into four equal groups: Group I was the control group that received a standard diet with tap water and were kept in their rearing cages without exposure to benzene and quercetin. Group II (BZN vapour inhaled group) received the standard diet with tap water. They were exposed to 4000 ppm/kg BW benzene vapours through cotton soaked with benzene hanged directly above their cage for 4 hrs./ day for 5 days /week with whole-body exposure for 6 weeks[14] . Group III (QCT) rats received a standard diet containing 0.3% powdered quercetin and were provided with water along the experiment time. Group IV (BZN-QCT) received a normal diet mixed with 0.3% powdered quercetin along with the experiment. They were provided with water and exposed to benzene inhalation.

2.4 Blood Collection

At the end of the 6th week, the rats were anesthetized by a single dose of xylazine-ketamine combination (1:9) intramuscular injection. Blood samples were collected by withdrawing the blood by heart puncture. Collected blood was centrifuged at 3000 rpm for 15 min and the sera were subjected to biochemical analysis.

2.5 Biochemical Assessments

COBAS INTEGRA 400 plus system - fully automated biochemical analyser (Germany) was used to determine the concentration of the following biochemical markers in rats' sera of control and experimental grouped rats. The activity of alanine (ALT), aspartate (AST) aminotransferase and alkaline phosphatase (ALP) in serum was assayed by the commercial kits (NS, BIOTECH Co., Egypt). In this study, kidney function was assessed by measuring blood urea and serum creatinine levels. Serum urea was determined using the diacetyl monoxime method and serum creatinine was determined through the alkaline picrate method (Jaffe's Method) by using a commercial kit (BIOLABO, France) [15].

2.6 Determination of Sperm Characteristics

The caudal part of the epididymis was isolated and weighed. It was then dispersed in 2ml of phosphate buffer solution (PBS, pH 7.2) at 37℃. After 10 min, it was collected in a tube and used to analyse sperm motility, concentration, viability and abnormalities [16].

2.6.1. Sperm Motility

A drop of sperm suspension was used to evaluate sperm motility. The number of 200 motile and non-motile spermatozoa were observed using an Olympus microscope. Sperm motility refers to the forward movement of sperm, and in situ motility is not calculated [17].

2.6.2. Sperm Concentration

Sperm concentration was determined by the Neubauer hemacytometer slide, based on the WHO laboratory manual for the examination and processing of human semen [18].

2.6.3. Sperm Viability Percentage

Sperm viability was determined by using eosin-nigrosin staining in a 3% sodium citrate dehydrated solution, according to the method described by Ajayi and Akhigbe [19].

2.6.4. Sperm Abnormalities

About 100 spermatozoa were observed under a microscope for variations in sperm morphology [20].

2.7 Histological Analysis

Lungs were excised then cut into smaller cubes (about 0.5 cm^3) and preserved in 10% formalin. Following was the process of histological preparation: the organs were fixed in formalin fixative for 24 hours to harden the tissue and inactivate enzymes that might otherwise degrade the tissue. The tissues were then dehydrated through ascending concentrations of ethanol (50%, 70%, 95% and 100%), each for 1 hour. Next they were cleared in xylene three times, each for 1 hour, and were later infiltrated in paraffin wax also three-time each for ½ hour in the oven at 60℃. Finally they were embedded in paraffin wax. Sections were cut at 5µm thickness with a rotary microtome. The sections were stained by haematoxylin and eosin stains [21].

2.8 Statistical Analysis

One-way analysis of variance was used for statistical analysis (ANOVA) and post hoc Tukey's multiple comparisons tests using GraphPad Software 7.01 (2016). Data was expressed as means ± standard deviation (SD), and $p<0.05$ was considered the minimum significant level.

3. Results

The present study results show that exposure to benzene and oral administration of quercetin and their combinations for six weeks did not produce any mortality in Wistar rats.

3.1 Biochemical Results

The results of liver and kidney function evaluation are shown in Tables 1 and 2. From the current findings, a significant ($P<0.01$) increase in serum ALP level (400.20 ± 15.64 U/L) in the BZN group was observed in comparison to the control group (235.50 ± 8.47 U/L). While AST (160.00 ± 1.22 U/L) and ALT (78.00 ± 2.04 U/L) levels increased insignificantly ($P>0.05$) in comparison to control rats (AST: 140.30 ± 11.82; ALT: 72.50 ± 3.70 U/L). However, AST (140.00 ± 5.54), ALT (73.25 ± 2.25), and ALP (329.90 ± 14.02) levels of the liver insignificantly ($P>0.05$) decreased in the BZN-QCT group (140.00±5.54; 73.25±2.25; 329.90±14.02, respectively) as compared to the BZN group (160.00±1.22; 78.00±2.04; 400.20±15.64, respectively). Moreover, the levels of AST and ALT in the BZN-QCT group almost reached near the control group level.

Table 1: Effects of benzen and quercetin on the activity of liver enzymes in treated and control groups

Groups

Parameters	Control Mean ± SD	BZN Mean ± SD	QCT Mean ± SD	BZN-QCT Mean ± SD
AST (U/L)	140.30 ± 11.82	160.00 ± 1.22	123.00 ± 5.88$^{\#}$	140.00 ± 5.54
ALT (U/L)	72.50 ± 3.70	78.00 ± 2.04	68.25 ± 4.90	73.25 ± 2.25
ALP (U/L)	235.50 ± 8.47	400.20 ± 15.64**	191.20 ± 13.59$^{\#\#\#}$	329.90 ± 14.02$^{!}$

** for p<0.01 (vs. control group); $^{\#}$, $^{\#\#\#}$ for p<0.05, p<0.001, respectively (vs. BZN group); while $^{!}$ for p<0.05 (vs. QCT group).

Exposure to benzene caused an insignificant (P>0.05) rise in blood urea and serum creatinine levels compared to the control group (Table 2). In the group that was exposed to both benzene and quercetin, the effects of benzene was partially reversed as seen in the blood urea and serum creatinine levels in comparison to the rats exposed to benzene alone (46.50±0.95; 0.35 ± 0.02, respectively).

Table 2: Effects of benzene and quercetin on the level of kidney function parameters

	Groups			
Parameters	**Control Mean ± SD**	**BZN Mean ± SD**	**QCT Mean ± SD**	**BZN-QCT Mean ± SD**
Blood Urea (mg/dL)	45.00 ± 1.95	50.50 ± 2.53	37.00 ± 2.79$^{\#\#}$	46.50 ± 0.95$^{!}$
Serum Creatinine (mg/dL)	0.35 ± 0.02	0.37 ± 0.02	0.32 ± 0.02	0.35 ± 0.02

$^{\#\#}$ for p<0.01 (vs. BZN group); while $^{!}$ for p<0.05 (vs. QCT group).

3.2 Sperm Quality Analysis

Assessment of various sperm parameters showed a difference in sperm concentration, motility, viability and abnormality between control and treated groups (Table 3).
Relative to the control group (count: 45.78±5.62x10^{6}/epididymis; motility: 88.75±6.34%; viability: 91.25±2.63%; abnormality: 6.25±1.25%), the animals that were exposed to benzene inhalation showed an insignificant (p>0.05) decrease in the total sperm count, sperm viability percentage, and significantly (p<0.05) decreased sperm motility as well as a significant (p<0.05) increase in the rate of morphologically abnormal sperms was detected (count: 40.05±4.44x10^{6}; motility: 72.25±4.99%; viability: 88.75±3.86%; abnormality: 12.25±2.50%). In the group that was exposed to both benzene and quercetin, the effects of benzene partly reversed as seen in the total sperm count, motile sperms and percentage of sperm viability, with a significant decrease in the rate of abnormal sperm in comparison to the rats exposed to benzene only (count: 43.48±3.54x10^{6}; motility: 77.50±9.57%; viability: 91.25±2.21%; abnormality: 10.25±2.63%).

Table 3: Sperm quality analyses of rats in the control and treated groups

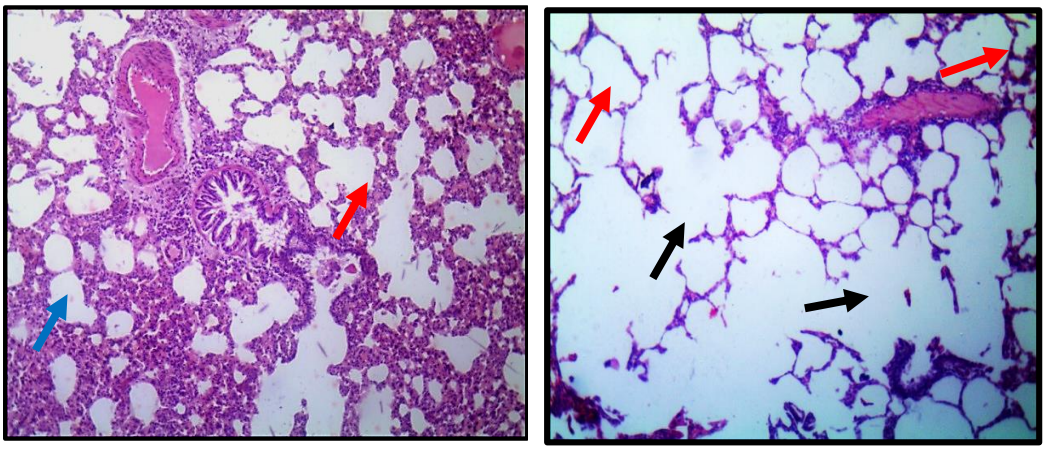
	Groups			
Parameters	**Control Mean ± SD**	**BZN Mean ± SD**	**QCT Mean ± SD**	**BZN-QCT Mean ± SD**
Sperm Count (10^{6}/epididymis)	45.78 ± 5.62	40.05 ± 4.44	49.71 ± 6.69	43.48 ± 3.54
Sperm Motility %	88.75 ± 6.34	72.25 ± 4.99*	90.50 ± 5.44$^{\#}$	77.50 ± 9.57
Sperm Viability %	91.25 ± 2.63	88.75 ± 3.86	91.75 ± 3.30	91.25 ± 2.21
Sperm Abnormality %	6.25 ± 1.25	12.25 ± 2.50**	4.25 ± 1.25$^{\#\#\#}$	10.25 ± 2.63$^{!}$

*, ** for p<0.05, p<0.01, respectively (vs. control group); #, ### for p<0.05, p<0.001, respectively (vs. BZN group); while ! for p<0.05 (vs. QCT group).

3.3. Histological Effects

Figure 1(a) illustrates the lung of the control group, in which the alveolar sacs, alveolar ducts, and alveoli are all present in a normal pulmonary tissue architecture with clear patent

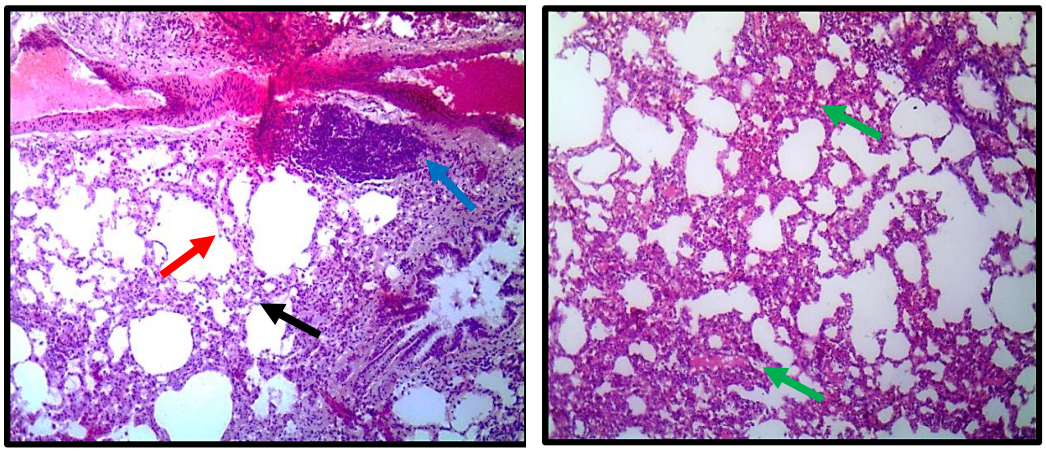
bronchial airways and alveolar cavities, including the alveolar sacs, alveolar ducts and alveoli. The pulmonary parenchyma had a normal distribution of pulmonary vessels. The thickness of the alveolar septa was normal and there were no abnormalities in the alveolar septal blood capillaries. Our results showed that benzene inhalation affected the histological structure of the lung in the treated rats (Figure 1(b)) in which dilation in the alveolar sac and thinning of alveolar wall which are abnormal features for the lung and also emphysema which is thinning of the alveolar sac wall and broken in someplaces to be larger alveoli than normal and dilation in the blood vessel with high congestion between the alveolar sacs and more elasticity in smooth muscles walls.



(a) **(b)**

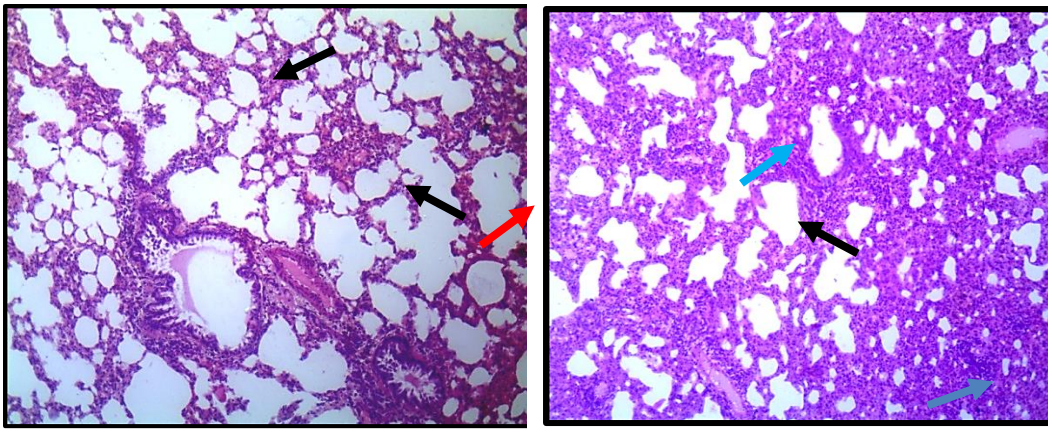
Figure 1: Section through the lungs (a) Section through the lung of the control group showing normal alveolus [14] and alveolar sac [14], 100x (b) Section through the lung of BZN exposed group showing Emphysema [14], and thinning of the wall of the alveolar, H&E,100x.

While Figure 2(a) shows other alterations in the histological structure of the lung in the benzene-treated rats, including highly lymphatic infiltration and thickening of the alveolar sac walls. Congestion between the alveolar sacs can also be seen in Figure 2(b). An extremely normal feature of the alveolar sac and whole lung tissue structure is visible in Figure 3(a), which includes the quercetin received group meaning that no side effects of this substance when administrated alone. On the other hand, quercetin has some ameliorate action in the lung of treated rats (Figure 3(b)) where normal features of the lung were shown in the BZN-QCT group with some inflammatory cells. It may be due to the short duration of the experiment or the amount of the dose used in our investigation.



(a) **(b)**

Figure 2: Section through the lung (a) BZN exposed group showing alveolar sac [14] thickening in the alveolar wall [14], lymphatic infiltration [14], (b) Section through the lung of BZN exposed group showing congestion of red blood cells [14], H&E, 100x.



(a) **(b)**

Figure 3: Section through the lung of (a) QCT exposed group showed the extremely normal structure of alveolar [14], alveolar sac [14], (b): Section through the lung of BZN-QCT exposed group showed the good feature of [14], presence of inflammatory cells [14], H&E, 100x.

4. Discussion

In the current study, increased serum ALT, AST and ALP levels were observed which is in accordance with the results obtained by D'Andrea and Reddy [22] and Abd El-Shakour, et al. [23] that benzene intoxication increased liver enzymes (AST, ALT, and ALP) activities. Free radicals overcome the antioxidants, leading to damaging and deadly cellular impacts by oxidation of membrane phospholipids, DNA, cellular proteins and enzymes, thus shutting down cellular respiration [24]. Therefore, it can be assumed that exposure to benzene and its metabolites leads to its accumulation in the liver tissue, which eventually leads to lipid peroxidation of the membranes [25]. The oxidative stress and reduction in endogenous antioxidants lead to the release of AST, ALT and ALP [26]. Since the reactive oxygen species play an important role in liver toxicity, it was considered to evaluate the effects of antioxidants on liver enzymes. Modern studies have shown that quercetin has anti-inflammatory activity and has shown it to be a powerful antioxidant [27-29]. In the present study, changes in liver enzymes activities were observed in the BZN-QCT group following exposure of animals to benzene, which is in agreement with previous reports [30].

Inhalation of benzene had a negative impact on kidney function parameters. There was a nonsignificant increase in creatinine and urea levels synchronized with increasing the duration of exposure to benzene. This outcome is consistent with the findings of Neghab, et al. [31]. Kidney function is the formation of urine. In the process many chemicals and toxic substances that are concentrated in the nephrons, are excreted by kidneys. Prolonged exposure to these chemicals leads to damage to the internal tissues since kidney is the main organ to excrete benzene metabolites [32]. The nephrotoxicity of benzene was recorded previously with an increase in serum creatinine (+30%) and blood urea nitrogen (+33%) in rats exposed to benzene at a dose of 800 mg/kg in a span of 30 days [33].

In this study, the benzene-induced kidney biomarkers change associated increase in creatinine and serum urea levels in the rat serum reduced non-significantly in the BZN-QCT group. The results of the current research are supported by the findings from previously published studies where quercetin has been reported to show some protective effects on renal injury in rats [34]. Also, quercetin has previously been reported to alleviate the cisplatin-induced oxidative stress in the rat kidneys [35]. These previously published studies, together with the findings of the present study, indicate the protective effects of quercetin in chronic kidney disease rat models.

Disorders of the reproductive system have become a public health problem, as they may cause abortion and abnormal results in the offspring. In 20 percent of infertile cases, the problem was mostly among male and no clear etiological component had been detected in up to 40% of male cases [36]. Numerous epidemiological research has been conducted to study the link between the risk of male infertility and exposure to chemical solvents. Current findings support other studies which revealed that the likelihood of male infertility raised considerably due to occupational solvent exposure [37].

Solvents can have numerous effects on human fertility because of the volatile nature of these solvents, such as benzene, which can easily pass the blood-testis-barrier and cause damage to the spermatogenic cell line and affect sperm morphology and function [38]. It has been found that the quality of semen is abnormal in workers exposed occupationally to hydrocarbon solvents, including benzene, xylene and toluene. Semen abnormalities might include changes in viscosity, the capacity of liquefaction, sperm number, sperm morphology and sperm motility compared to the unexposed workers [39]. Correspondingly, solvent exposure may have a proportionate influence on human seminal quality with an exposure range [40]. The data further indicates that reduced sperm number, sperm motility and elevated aberrant sperm morphology are linked to worker exposures to benzene. This finding is in accordance with Rubes, et al. [41] who stated that exposures to air pollutants, including benzene by-products, might be associated with the decreased quality of semen in traffic policemen. Results of this study show that benzene exposure produces significant changes in both sperm motility and sperm abnormality. It is widely known that sperm motility significantly depends on the continuous production of ATP through oxidative routes. In this metabolism, many oxidative enzymes are required to be active in the mid-piece of the sperm.

Medicinal herbs and their active principles have garnered considerable attention lately since they contain anti-prooxidant and antioxidant capabilities. Plant compounds have been identified to preserve via scavenging of free radicals, thus regulating toxicant detoxification and providing an antioxidant defence mechanism [42]. One of the most prevalent flavonoids in nature, quercetin, is a flavonoid found in a variety of fruits and vegetables [43]. It is known to have a variety of pharmacological effects like antitumor, antioxidant, anti-diabetic, anti-inflammatory, bacteriostatic and as being a powerful protectant against the reproductive damage caused by several substances [10]. Treatment with such antioxidant material increases sperm concentration, motility, viability and fertility while reducing the number of abnormal sperms. It has been suggested that quercetin causes androgen depletion at the target level, particularly in the cauda epididymis, thereby affecting the physiological maturation of sperm [44].

The lung is a vital organ for breathing and the organ, which gets all of the heart's output. The pulmonary vascular bed's broad surface also has an essential part in defending the body and controlling amounts of biologically active elements in the circulation. Chronic benzene

exposure is well recognized to irritate the respiratory tract and cause negative changes in respiratory function metrics and cellular morphology [45].

Studies on the toxic effects of benzene revealed similar results on different organs and tissues, including the respiratory system (nasal epithelium, trachea), in which different histological alterations were shown in treated animals and workers in histological techniques laboratories [46, 47]. Acute granular tracheitis, bronchitis, laryngitis, acute pulmonary oedema, and massive haemorrhages of the lungs have been recorded at the autopsy, and it has also been found that in some cases, benzene causes death if inhaled for a long time [23, 48].

There is significant experimental evidence that a redox imbalance plays a role in lung fibrogenesis. This chronic illness is typically unresponsive to standard anti-inflammatory and immunomodulatory treatment [49]. The respiratory effects of benzene have been already documented in humans, following inhalation of benzene vapours which produces mucous membrane irritation in workers and nasal irritation and sore throat in male and female workers [50].

The effects of exposure to benzene vapour on other systems, besides the respiratory system, were not found. After acute exposure to benzene vapours, respiratory effects have been detected in humans. 15 male workers employed in removing residual fuel from shipyard tanks were evaluated for benzene exposure [51]. During work-related exposure of 60 ppm for up to three weeks, 80 percent of employees were found to have mucous membrane irritation and 67 percent experienced dyspnoea. Male and female employees exposed to 33 and 59 ppm benzene for more than a year, respectively, complained of nasal discomfort and sore throat [52].

6. Conclusion

In conclusion, benzene inhalation causes alterations in the renal and liver function activity, spermatological parameters and architecture of lung tissues. Quercetin relieves the hazards associated with benzene inhalation. The present work suggests a beneficial effect of quercetin against benzene-induced hepatotoxicity, nephrotoxicity, reproductive toxicity and histological changes in lung tissues via improving the important studied biomarkers of these vital organs.

7. Conflict of Interest: The authors declare that they have no conflicts of interest.

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