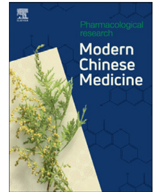
Pharmacological Research - Modern Chinese Medicine 7 (2023) 100257

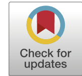
Contents lists available at ScienceDirect

Pharmacological Research - Modern Chinese Medicine

journal homepage: www.elsevier.com/locate/prmcm



Ferulic acid mitigates 2-methoxyethanol-induced testicular oxidative stress via combined downregulation of FoxO1, PTEN, and modulation of Nrf2-Hmox1-NQO1 signaling pathway in rats



Olubisi E. Adeyi[a], Oluwatobi T. Somade[a,b,*], Adewale S. James[c,d], Akindele O. Adeyi[e], Samuel N. Ogbonna-Eze[a], Olamilekan Q. Salako[a], Tomiwa V. Makinde[a], Oluwasogo M. Ajadi[a], Sefiat A. Nosiru[a]

[a] Department of Biochemistry, College of Biosciences, Federal University of Agriculture, Abeokuta, Nigeria
[b] Phytomedicine, Biochemical Toxicology and Biotechnology Research Laboratories, Department of Biochemistry, College of Sciences, Afe Babalola University, PMB, 5454, Ado-Ekiti 360001, Nigeria
[c] Department of Chemical Sciences, Augustine University, Ilara-Epe, Lagos P.M.B. 1010, Nigeria
[d] School of Biomedical Sciences, Morsani College of Medicine, University of South Florida, Tampa FL 33612, USA
[e] Animal Physiology Unit, Department of Zoology, University of Ibadan, Ibadan, Nigeria

University of Ibadan Library

A R T I C L E I N F O

Keywords:
Ferulic acid
2-methoxyethanol
Oxidative stress
Antioxidants

A B S T R A C T

Introduction: Ferulic acid (FERA) is a natural antioxidant that is richly found in herbs, including Ligusticum chuangxiong, Cimicifuga heracleifolia, and female ginseng (Angelica sinensis), which are utilized in modern Chinese medicine, and in cereals/grains including rice, which is mostly consumed by humans. 2METE on the other hand, is a ubiquitous substance that has many industrial applications, including use in the preparation of dyes for textiles, hydraulic fluid for automobiles, paints, and liquid soaps. It is a testicular toxin, which can induce oxidative stress in the testis of rats. Therefore, this study investigated the effect of FERA, which was concomitantly administered, against 2-methoxyethanol (2METE)-induced testicular oxidative stress in rats.
Methods: Male Wistar rats totaling twenty (20), separated into four (4) groups, were used for the study. Rats in group one served as the control, rats in groups two and three were administered 100 mg/kg of 2METE only for 30 consecutive days, but only rats in group three were concomitantly treated with 50 mg/kg of FERA for the same duration, while rats in group four were treated with 50 mg/kg of FERA only.
Results: Following analysis, 2METE administration caused a significant reduction in the relative testes weight (RTW), NAD(P)H quinone oxidoreductase 1 (NQO1), and reduced glutathione (GSH) levels, as well as superoxide dismutase (SOD) and glutathione S-transferase (GST) activities in the testis of rats compared with the control. Moreover, 2METE administration also significantly increased the testicular levels of malondialdehyde (MDA), nitric oxide (NO), and RNA gene expressions of heme oxygenase 1 (Hmox1), nuclear factor erythroid 2-related factor 2 (Nrf2), forkhead box protein O1 (FoxO1), and phosphatase and tensin homolog deleted on chromosome 10 (PTEN) compared with the control. FERA treatment, on the other hand, significantly decreased the testicular levels of MDA, as well as Nrf2, Hmox1, PTEN, and FoxO1 gene expressions, and significantly increased the testicular GSH and NQO1 levels, activities of GST, SOD, glutathione peroxidase (GPx), and catalase (CAT) compared with 2METE only administered rats.
Conclusion: 2METE-induced testicular oxidative stress, marked by the depletion of the endogenous antioxidant systems, was recorded, which resulted in the activation of PTEN, FoxO1, and Nrf2 genes in rats. FERA demonstrated a strong antioxidant effect by restoring the levels and activities of the endogenous antioxidants as well as downregulating the expressions of PTEN, FoxO1, and Nrf2 in the testis of rats.

* Corresponding author at: Department of Biochemistry, College of Biosciences, Federal University of Agriculture, Abeokuta, Nigeria.
E-mail address: somadeot@funaab.edu.ng (O.T. Somade).

https://doi.org/10.1016/j.prmcm.2023.100257
Received 7 April 2023; Received in revised form 1 May 2023; Accepted 5 May 2023
Available online 6 May 2023
2667-1425/© 2023 The Author(s). Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/)

1. Introduction

Oxidative stress caused by the cellular production of free radicals is known to affect the well-being of the reproductive system, which can eventually lead to testicular dysfunction [1]. Free radicals can attack the cells of the reproductive system, causing serious damage that can lead to perturbations and defects in the process of spermatogenesis [1]. Thus, the excessive formation of peroxidants and free radicals that overwhelm the antioxidant defense mechanisms can cause oxidative stress [2]. A balance between the produced reactive species and their prompt elimination is crucial for the normal function of the testicular cells. Failure to scavenge or repair the destructive effects of free radicals can cause serious damage to the testicular cells [3]. To ensure the wellness of cells, antioxidants can protect against free radical-induced cellular damage by stopping their formation or mopping up the already-produced free radicals. The body has several preventive endogenous antioxidants, which are non-enzymatic or enzymatic. Some of these systems include GST, catalase, GSH, SOD, and GPx [4–7].

METE belongs to the group of chemicals known as glycol ethers. These chemicals have many industrial applications, including use in the preparation of dyes for textiles, hydraulic fluid for automobiles, paints, liquid soaps, varnishes, anti-icing additives, leather finishing, lacquers, and the production of plastics for food packaging [8]. However, glycol ethers can induce toxicities in animals and humans and have been reported to cause endocrine system toxicity [9]. Of particular concern, 2METE has been reported to cause testicular, bone marrow, fetal, and thymus toxicities [10]. Specifically, 2METE has been found to induce pulmonary toxicity [11], testicular toxicity [12,13], hepatic toxicity [14,15], and renal toxicity [10] through oxidative stress. Additionally, 2METE exposure has been associated with testicular spermatotoxicity, hematotoxicity, testicular and thymocyte degenerations, developmental problems, and leucopenia [16]. Despite the wide industrial and domestic applications of 2METE and its potential health risks, there are still only a few reported therapeutic studies against 2METE-induced toxicities.

FERA, otherwise known as 4-hydroxy-3-methoxycinnamic acid, is a plant-derived phenolic acid compound and a caffeic acid derivative that is abundantly present in beer, coffee, fruits, and vegetables [17]. FERA is found in herbs, including Ligusticum chuangxiong, Cimicifuga heracleifolia, and female ginseng (Angelica sinensis), which are employed in traditional Chinese medicine (TCM) [17]. In Chinese, Chuanxiong rhizome (otherwise called CX) is the dried rhizome of Ligusticum chuanxiong Hort, a very popular edible medicinal herb traditionally known for its excellent therapeutic effect on cerebrovascular and cardiovascular diseases [18]. Also, it is a major soup ingredient that requires regular consumption due to its health benefits [18]. For Cimicifuga heracleifolia Kom, the dried rhizome is also well known in TCM due to its analgesic, antipyretic, and anti-inflammatory activities [19]. The root extract of another FERA-containing herb, Angelica sinensis, also referred to in TCM as danggui, has been utilized traditionally to treat various blood-associated disorders like uterine disorders, blood deficiencies, menstrual cramps, as well as ischemic brain and heart diseases [20,21]. In plants, FERA is biosynthesized from phenylalanine metabolism via the shikimate pathway. Naturally, FERA is a potent antioxidant, and this antioxidant property is conferred by the presence of an extended side chain that is conjugated and a phenolic nucleus in its chemical structure [17]. As a result, FERA can protect against cellular lipid peroxidation, and in some countries, it has been approved as an additive in foods [17]. Also, FERA's antioxidant, hepatoprotective, anti-allergic, anti-carcinogenic, antimicrobial, anti-inflammatory, antiviral, antithrombotic, and vasodilatory effects have been documented. In addition, FERA's ability to increase sperm viability, act as a cross-linking agent in food preservation, and protect against the sun in skin creams and sunscreens has been reported [17].

In this study, we checked the protective effect of FERA and its probable mechanism of action against 2METE-induced oxidative stress in the testis of rats.

2. Materials and methods

2.1. Chemicals, administered substances, and primers

The 2METE used ($C_3H_8O_2$; 99.5% purity) was packaged by BDH Laboratory Supplies, England. FERA ($C_{10}H_{10}O_4$; 95% purity; CAS: 1135–26–6) was manufactured and ordered from AK Scientific, USA. Other chemicals used, including Ellman's reagent, trizol, sodium chloride, glacial acetic acid, adrenaline, hydrogen peroxide, trichloroacetic acid, hydrochloric acid, etc., were of analytical grade. The forward and backward sequences of primers for Hmox1, NQO1, FoxO1, Nrf2, PTEN, and β-actin were ordered from ShineGene Corporation, Shanghai, China.

2.2. Experimental animal and the study design

Male Wistar rats (n = 20; 200 g) were used in this study. They were housed in a suitable environment and had access to potable drinking water and rat feed. Approval to conduct the study was obtained from the local Institutional Animal Care and Use Committee (IACUC) of the Department of Biochemistry, FUNAAB, with approval number FUNAAB-BCHREC 998,460 – IDRD2022/073. After acclimatization for a month, the 20 rats were separated into 4 groups. Rats in group I served as the control and were not administered any test substances. Animals in groups II and III were orally given 100 mg/kg of 2METE [15] for 30 consecutive days, but only group III was simultaneously treated with 50 mg/kg of FERA [22] orally for 30 consecutive days, while animals in group IV were only administered 50 mg/kg of FERA for 30 days.

2.3. Sacrifice, tissue harvest, and processing

At the end of all administration, rats were sacrificed 24 h later. The rats were handled humanely following documented guidelines for the handling and utilization of research animals [14]. The testis samples were dissected and harvested. Each testis was rinsed in ice-cold normal saline (9.0 g/L of NaCl), dried on filter paper, and weighed. Portions of each testis were excised and homogenized in 0.1 M phosphate (pH 7.4) buffer. The obtained homogenates were separated using a centrifuge, and the resulting supernatants were separated and used to determine the level or activity of the oxidative stress markers. In addition, portions of the testis from each rat were collected in Trizol reagent for RNA preservation and extraction, and in formalin (10%) for histopathological analysis.

2.4. Biochemical assays

2.4.1. Estimation of testicular MDA, NO, and GSH concentrations

The testicular concentration of MDA was estimated using the method described by Katerji et al. [23]. The reaction mixture comprised of 0.1 mL of testis sample and 2 mL of equal volume of hydrochloric acid (HCl)-thiobarbituric acid (TBA)-trichloroacetic acid (TCA) reagent, was boiled for 15 min, and cooled thereafter. This was followed by centrifugation for 10 min at 3000 rpm, and the absorbance of the separated supernatant was determined at 532 nm. The testicular concentration of NO was estimated using the method described by Chen et al. [24]. The reaction mixture, which was made up of a testis sample (0.05 mL), distilled water (0.1 mL), and sulfanilamide (0.15 mL), was incubated for 10 min. After incubation, 0.15 mL of N-naphthyl ethylenediamine was added and the incubation was repeated for 10 min. NO concentration was then measured at 540 nm spectrophotometrically, while the testicular concentration of GSH was estimated using the method described by Begum et al. [25]. Briefly in that method, an equal volume of sulphursalicyclic acid and sample (testis) was mixed and centrifuged for 5 min at 3000 rpm. The supernatant (0.5 ml) was pipetted into a tube containing 0.1 M (pH 7.4) phosphate buffer (4 mL) and Ellman's reagent (0.5 mL). The absorbance of this mixture was determined at 412 nm.

University of Ibadan Library

Table 1
Reverse and forward primer sequence of interested genes.

Interested genes		Sequences
FoxO1	Reverse	CTTGCCTCCCTCTGGATTGA
	Forward	CGGCCCCCAATCTCGG
PTEN	Reverse	ACCTTTAGCTGGCAGACCAC
	Forward	AGACCATAACCCACCACAGC
Hmox1	Reverse	GGGTTCTGCTTGTTTCGCTC
	Forward	GGCTTTAAGCTGGTGATGGC
Nrf2	Reverse	GCAAGCGACTCATGGTCATC
	Forward	CAGCATGATGGACTTGGAATTG
NQO1	Reverse	TCTGCGTGGGCCAATACAAT
	Forward	AGCGCTTGACACTACGATCC
β-Actin	Reverse Forward	CATCGGTAGGTCCGACACAA CCCGCGAGTACAACCTTCTT

2.4.2. Estimation of testicular GPx, SOD, GST, and cat activities

The testicular GPx activity was estimated using the method described by Katerji et al. [23]. In this method, hydrogen peroxide (0.1 mL), phosphate buffer (0.5 mL), reduced glutathione (0.2 mL), sodium azide (0.1 mL), testis sample (0.5 mL), and distilled water (0.6 mL) were added together and incubated for 3 min. Trichloroacetic acid (0.5 mL) was added, and the mixture was centrifuged. The resulting supernatant (1 mL) was added to a tube that comprised Ellman's reagent and dipotassium hydrogen phosphate, followed by the determination of absorbance at 412 nm. The testicular activity of GST was estimated using the method described by Abdulmalek et al. [26]. Briefly, reduced glutathione (0.03 mL), testis sample (0.03 mL), phosphate buffer (2.79 mL; 0.1 M; pH 6.5), and 2,4-dinitrochlorobenzenne (0.15 mL) were mixed. The absorbance of the mixture was determined at 340 nm for 3 min, at minute intervals. The testicular activity of CAT was estimated using the method described by Sinha [27]. In this method, 1 mL of hydrogen peroxide, 1.25 mL of 0.01 M (pH 7.0) phosphate buffer, and 0.25 mL of testis sample were mixed. The reaction of CAT present in the sample and hydrogen peroxide was stopped in a tube that contained 0.5 mL of dichromate-acetic acid reagent (1 ratio 3 respectively) at 0, 60, 120, and 180 s. After the mixture was subjected to heat in boiling water and cooled on ice, the absorbance was determined spectrophotometrically at 570 nm, while the testicular activity of SOD was estimated using the method described by Somade et al. [27]. Briefly in that method, a testis sample (0.2 mL) was added to a mixture of adrenaline (0.3 mL) and sodium bicarbonate buffer (0.05 M; pH 10.2). They were thoroughly mixed, and absorbance was determined at 480 nm.

2.4.3. Extraction of testis RNA, cDNA synthesis, gene amplification, and gene expression analyses

The testis samples homogenized in Trizol reagent were used for total RNA extraction. After RNA extraction and purification, cDNA was chemically synthesized from the extracted RNA using reverse transcriptase enzyme. The synthesized cDNAs were then amplified using the PCR technique to produce multiple copies of the target genes, using both the backward and forward primer sequences shown in Table 1. Each amplified gene was run on an agarose gel, the migrated bands were captured and the intensity of each band was quantified [15]. The relative expression of each gene was calculated with respect to the expression of the β-actin gene, which served as the housekeeping gene.

2.4.4. Histopathology

The 10% formalin-fixed sections of the testis were rinsed in ice-cold phosphate (pH 7.4) buffer for 12 h. After the dehydration step, the testis samples were embedded in paraffin, sectioned, and stained with hematoxylin-eosin dye. The microscopic slides were viewed under a microscope at a magnification of x400.

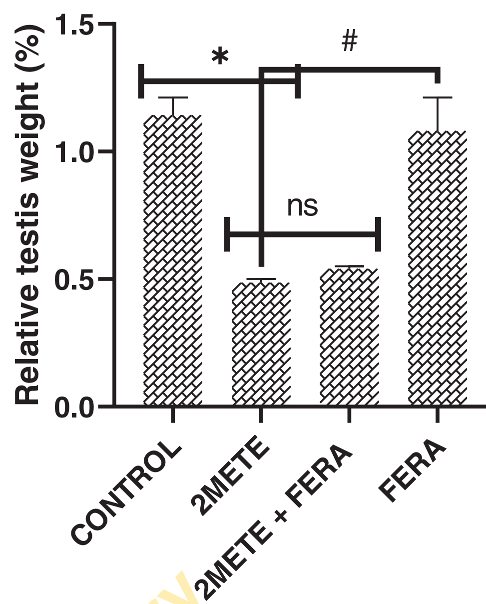


Fig. 1. Effect of FERA treatments on RTW of 2METE administered rats. Each of the bars stands for mean ± standard error of mean. *Significantly different compared with control ($p < 0.05$); #significantly different compared with 2METE only ($p < 0.05$). 2METE = 2 methoxyethanol; FERA = ferulic acid.

2.5. Statistical analyses

Statistical analyses were performed using GraphPad Prism (v8.0.0) for Windows, GraphPad Software, San Diego, California USA [28]. For each biochemical assay, the data gathered were analyzed using ANOVA, followed by Tukey's test to determine the level of significance among the four groups (n = 5). Results were presented as mean ± S.E.M, and only p-values not above 0.05 were considered significant.

3. Results

3.1. Effect of FERA treatment on RTW of 2METE exposed rats

Fig. 1 shows the results of the RTW of rats. Administration of 2METE to rats significantly decreased their RTW compared to the control group. Co-administration of FERA with 2METE (2METE + FERA) did not have a significant effect on the RTW compared to rats administered with 2METE alone.

3.2. Effect of FERA treatment on testis MDA level of 2METE exposed rats

In Fig. 2, the concentration of MDA was significantly elevated in rats administered with 2METE compared to the control group. However, co-administration of FERA with 2METE (2METE + FERA) significantly lowered the level of MDA compared to rats administered with 2METE alone.

3.3. Effect of FERA treatment on testis no level of 2METE exposed rats

Fig. 3 shows that the testicular NO concentration was significantly elevated in rats administered with 2METE compared to the control group. Co-administration of FERA with 2METE (2METE + FERA) did not have a significant effect on the testicular NO concentration compared to rats administered with 2METE alone.

3.4. Effect of FERA treatment on testis GSH level of 2METE exposed rats

In Fig. 4, the testicular GSH concentration was significantly lowered in rats administered with 2METE compared to the control group. How-

University of Ibadan Library

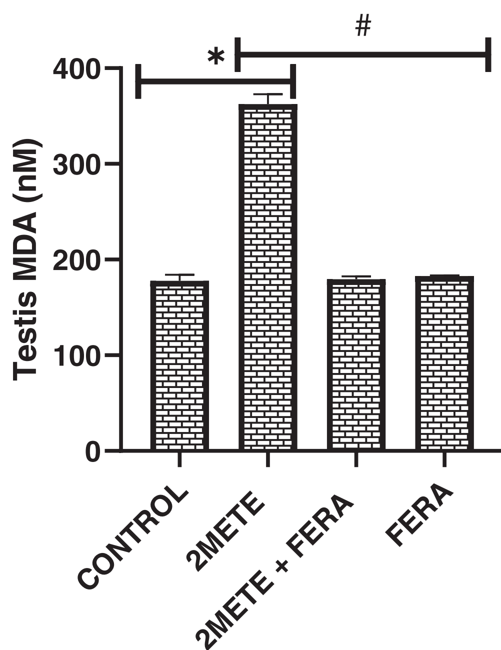


Fig. 2. Effect of FERA treatments on testis MDA concentration in 2METE administered rats. Each of the bars stands for mean ± standard error of mean. *Significantly different compared with control ($p < 0.05$); #significantly different compared with 2METE only ($p < 0.05$). 2METE = 2 methoxyethanol; FERA = ferulic acid.

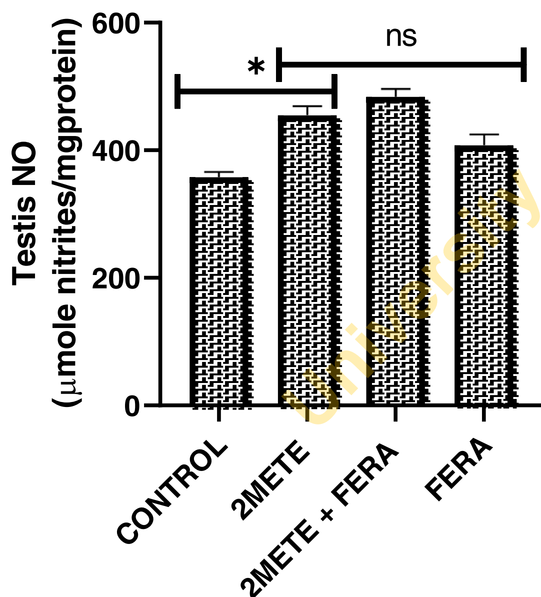


Fig. 3. Effect of FERA treatments on testis NO concentration in 2METE administered rats. Each of the bars stands for mean ± standard error of mean. *Significantly different compared with control ($p < 0.05$); #significantly different compared with 2METE only ($p < 0.05$). 2METE = 2 methoxyethanol; FERA = ferulic acid.

ever, co-administration of FERA with 2METE (2METE + FERA) significantly increased the concentration of GSH compared to rats administered with 2METE alone.

3.5. Effect of FERA treatment on testis GPx activity of 2METE exposed rats

In Fig. 5, there was a non-significant reduction in the testicular GPx activity following the administration of 2METE only to rats compared

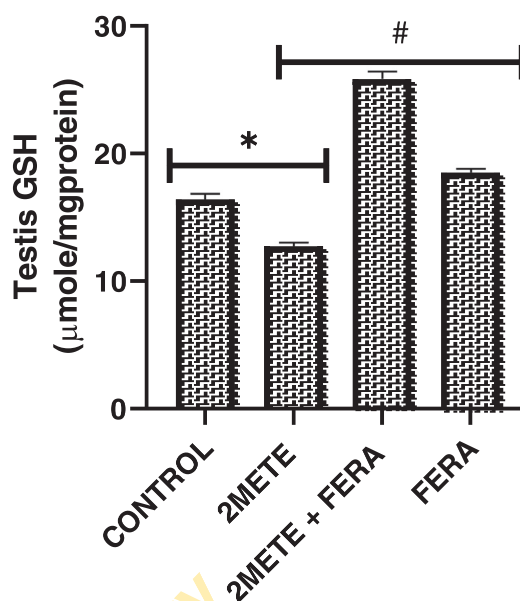


Fig. 4. Effect of FERA treatments on testis GSH concentration in 2METE administered rats. Each of the bars stands for mean ± standard error of mean. *Significantly different compared with control ($p < 0.05$); #significantly different compared with 2METE only ($p < 0.05$). 2METE = 2 methoxyethanol; FERA = ferulic acid.

University of Ibadan Library

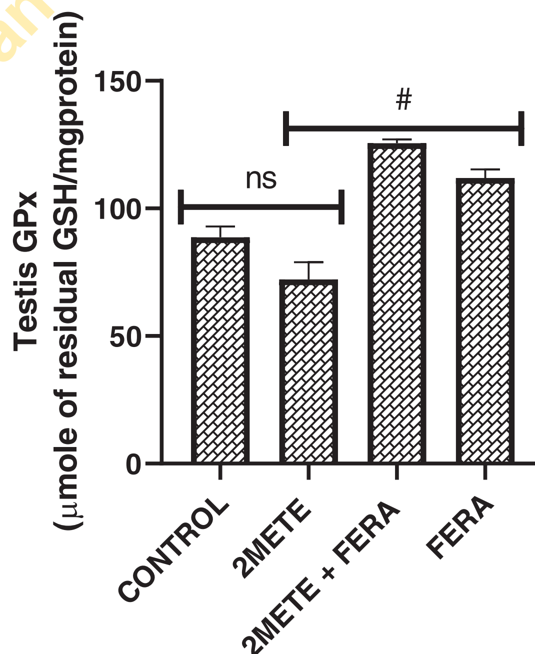


Fig. 5. Effect of FERA treatments on testis GPx activity in 2METE administered rats. Each of the bars stands for mean ± standard error of mean. *Significantly different compared with control ($p < 0.05$); #significantly different compared with 2METE only ($p < 0.05$). 2METE = 2 methoxyethanol; FERA = ferulic acid.

with the control group. However, FERA treatments (2METE + FERA) significantly raised the testicular activity of GPx compared with 2METE-only administered rats.

3.6. Effect of FERA treatment on testis GST activity of 2METE administered rats

Testicular GST activity was significantly lowered in rats treated with 2METE compared to control rats (Fig. 6). However, co-treatment with

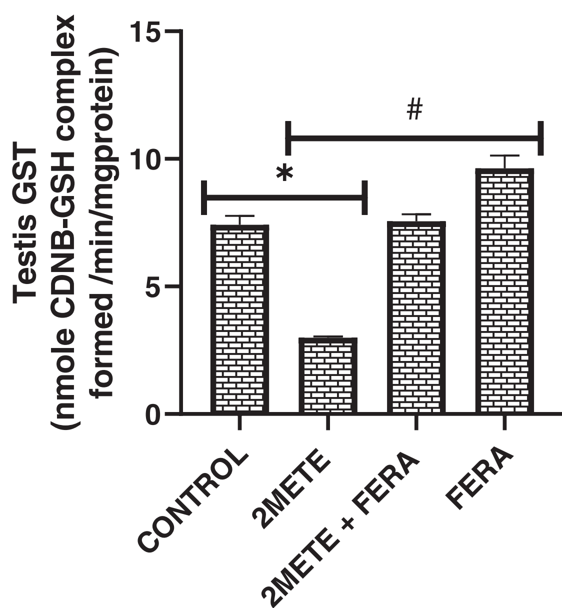


Fig. 6. Effect of FERA treatments on testis GST activity in 2METE administered rats. Each of the bars stands for mean ± standard error of mean. *Significantly different compared with control ($p < 0.05$); #significantly different compared with 2METE only ($p < 0.05$). 2METE = 2 methoxyethanol; FERA = ferulic acid.

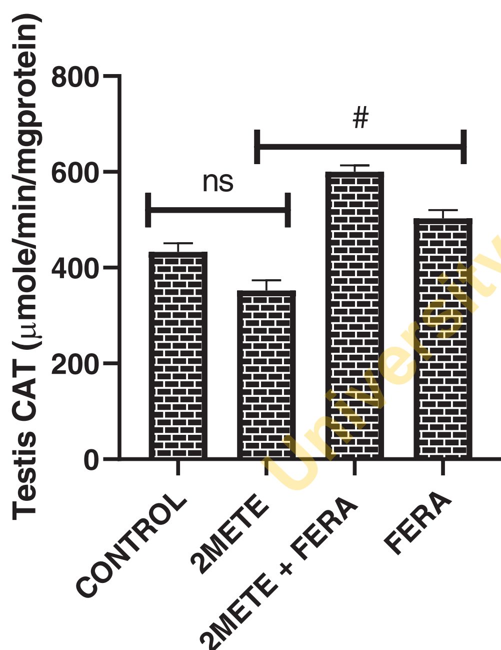


Fig. 7. Effect of FERA treatments on testis CAT activity in 2METE administered rats. Each of the bars stands for mean ± standard error of mean. *Significantly different compared with control ($p < 0.05$); #significantly different compared with 2METE only ($p < 0.05$). 2METE = 2 methoxyethanol; FERA = ferulic acid.

FERA (2METE + FERA) significantly elevated the activity of GST compared to rats treated with 2METE alone.

3.7. Effect of FERA treatment on testis cat activity of 2METE exposed rats

Fig. 7 shows a non-significant reduction in testicular CAT activity in rats treated with 2METE alone compared to control rats. However, co-treatment with FERA (2METE + FERA) significantly elevated the testicular CAT activity compared to rats treated with 2METE alone.

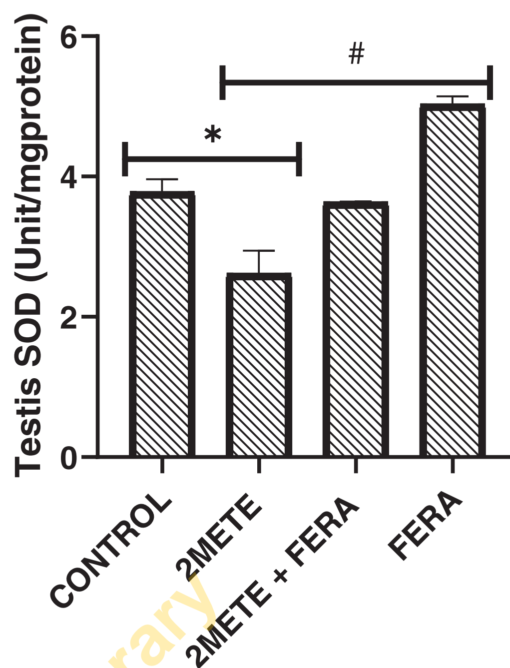


Fig. 8. Effect of FERA treatments on testis SOD activity in 2METE administered rats. Each of the bars stands for mean ± standard error of mean. *Significantly different compared with control ($p < 0.05$); #significantly different compared with 2METE only ($p < 0.05$). 2METE = 2 methoxyethanol; FERA = ferulic acid.

3.8. Effect of FERA treatment on testis sod activity of 2METE administered rats

Testis SOD activity was significantly decreased by 2METE administrations compared with the SOD activity recorded for the control rats (Fig. 8). FERA treatments (2METE + FERA) significantly elevated the activity of SOD compared with 2METE-only exposed rats.

3.9. Effect of FERA treatment on testis Nrf2, hmox1 and NQO1 relative gene expressions in 2METE exposed rats

Fig. 9 shows the results of testicular Nrf2, Hmox1, and NQO1 relative gene expressions. The relative gene expressions of both Nrf2 and Hmox1 in the testis were significantly elevated, while that of NQO1 was significantly reduced in rats treated with 2METE compared to the control group. However, co-administration of FERA and 2METE (2METE + FERA) significantly reduced the relative gene expressions of both Nrf2 and Hmox1, while it significantly elevated the NQO1 relative gene expression compared to rats treated with 2METE alone.

3.10. Effect of FERA treatment on testis FoxO1 and PTEN relative gene expressions in 2METE exposed rats

Fig. 10 represents the results of testicular FoxO1 and PTEN relative gene expressions. The relative gene expressions of both FoxO1 and PTEN in the testis were significantly elevated in rats treated with 2METE compared to the control group. However, co-administration of FERA and 2METE (2METE + FERA) significantly lowered the relative gene expressions of both compared to rats treated with 2METE alone.

3.11. Effect of FERA treatment on the testicular architecture of 2METE exposed rats

Administration of 2METE to rats caused the degeneration of seminiferous tubules, attenuation of germinal epithelium, and vacuolation of Sertoli cells compared to the control group, whose testes showed no

University of Ibadan Library

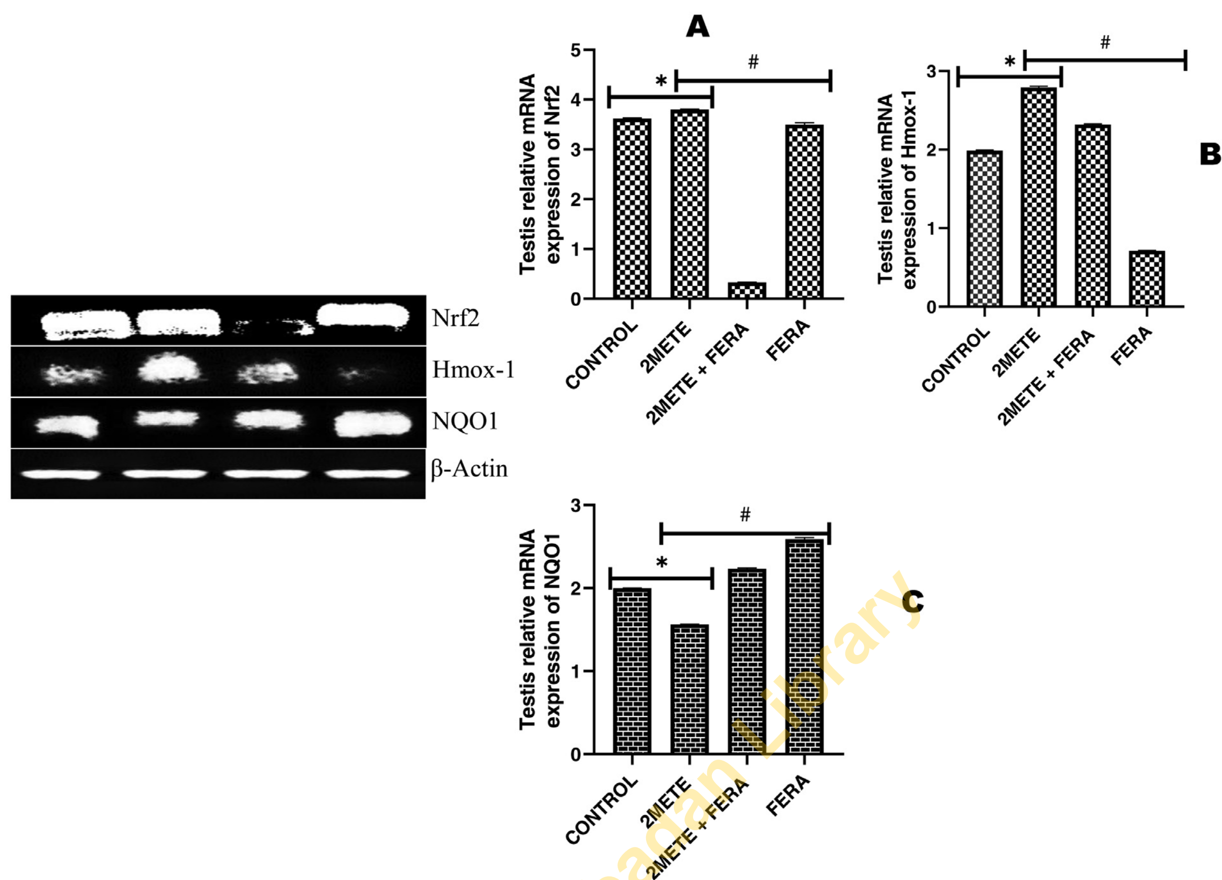


Fig. 9. Effect of FERA treatments on testis relative gene expressions of Nrf2 (A), Hmox1 (B) and NQO1 (C) in 2METE administered rats. Each of the bars stands for mean ± standard error of mean. *Significantly different compared with control ($p < 0.05$); #significantly different compared with 2METE only ($p < 0.05$). 2METE = 2 methoxyethanol; FERA = ferulic acid.

University of Ibadan Library

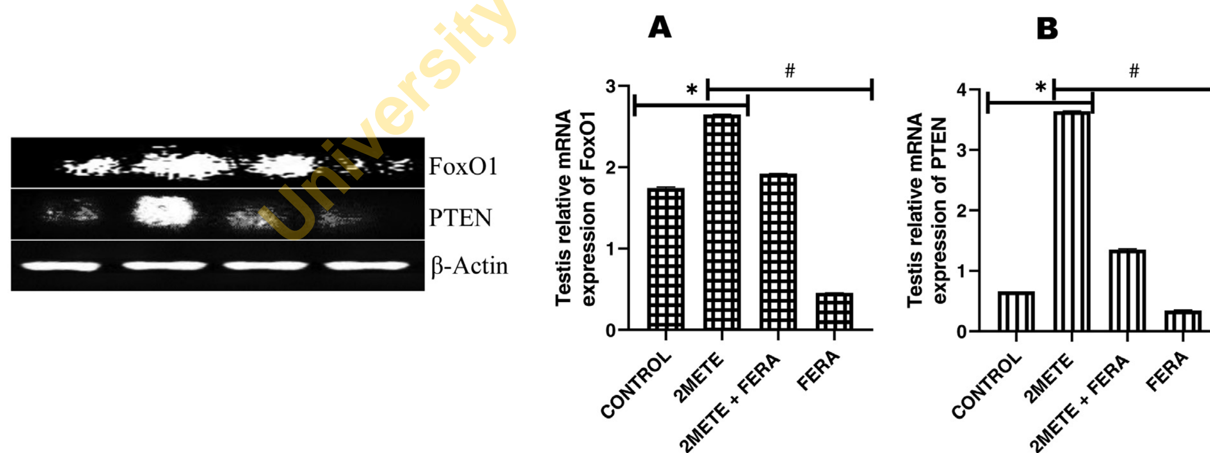


Fig. 10. Effect of FERA treatments on testis relative gene expressions of FoxO1 (A) and PTEN (B) in 2METE administered rats. Each of the bars stands for mean ± standard error of mean. *Significantly different compared with control ($p < 0.05$); #significantly different compared with 2METE only ($p < 0.05$). 2METE = 2 methoxyethanol; FERA = ferulic acid.

observable lesions (Fig. 11). Treatment with FERA (2METE + FERA) did not effectively correct these pathological anomalies, as there were still degenerated seminiferous tubules and vacuolated Sertoli cells in the testes of these rats (Fig. 11). Only the testes of rats in the 2 groups administered 2METE (groups 2 and 3) were significantly damaged compared with the testes of rats in groups (groups 1 and 4) not administered the toxicant (2METE). The testicular cells that were damaged significantly include the seminiferous tubule, epithelium, and Sertoli in group 2 (2METE only), while only the seminiferous tubule and Sertoli were significantly damaged in group 3 (2METE + FERA).

4. Discussion

Shiau-feng-saan and Dang-guei-nian-tong-tang are two Chinese medicinal preparations that are made up of 13 and 15 herbs, respectively [29]. Shiau-feng-saan, which contains Angelicae sinensis Radix,

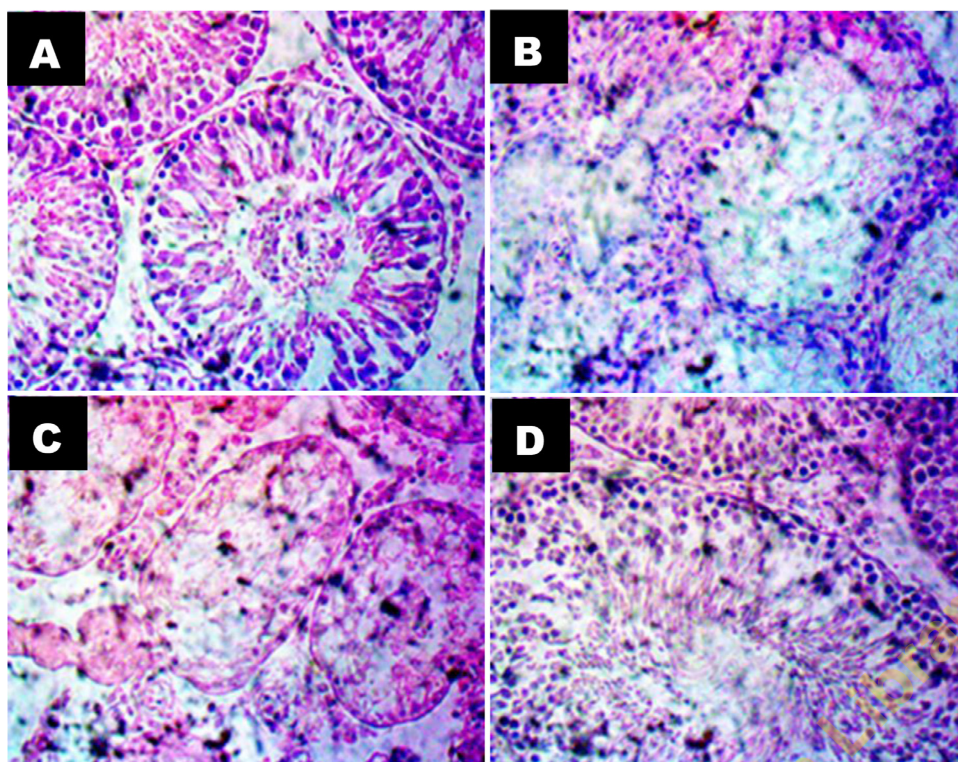


Fig. 11. Effect of FERA treatments on testicular cell architectures in 2METE administered rats (x 400 magnification). Control (A) shows is no observable lesion; 2METE only (B) shows the degeneration of seminiferous tubules, attenuation of germinal epithelium and vacuolation of sertoli cells; 2METE + FERA (C) shows the degeneration of seminiferous tubules and vacuolation of sertoli cells; while FERA only (D) = shows no observable lesion.

University of Ibadan Library

a FERA-containing herb, has been used to treat medica mentosa dermatitis, rubeola, urticaria, and eczema [29]. Similarly, Dang-guei-niantong-tang, which also contains Angelicae sinensis Radix and Cimicifugae Rhizoma, 2 FERA-containing herbs, is used to treat rheumatoid arthritis, sciatic neuralgia, rheumatic arthritis, and waist soft-tissue strain [29]. FERA is one of the bioactive agents in Angelica sinensis (Danggui) and Cimicifuga heracleifolia, and possesses numerous pharmacological properties such as anti-Alzheimer's, antithrombotic, anticardiovascular, anti-tumor, immunity enhancing, antibacterial, anti-inflammatory and anti-oxidative stress effects. Thus, this study investigated the mechanism through which FERA prevents 2METE-induced oxidative stress, by studying the involvement of some oxidative stress-associated genes in the testis of rats.

Oxidative stress is a cellular condition characterized by a depleted endogenous antioxidant system due to excessive and continuous cellular production of free radicals that cells can no longer curtail or manage [13]. Chemical-induced toxicity is a major consequence of cellular oxidative stress [27,30]. Therefore, this study checked the anti-oxidative stress effect of FERA on the testes of rats sub-acutely exposed to 2METE, which has been reported as a potent gonadotoxic agent in previous studies [9,12,13]. Our findings revealed a significant reduction in the RTW of rats administered with 2METE, which is consistent with previous studies. However, FERA treatments did not have a significant effect on the RTW of rats, which may be due to the concomitant administration of both test substances throughout the study. This finding is similar to the result obtained in another study by Somade et al. [13], where syringic acid was ineffective in protecting the testicular cells of rats against 2METE-induced reduction in RTW.

The susceptibility of sperm and testis cell membranes to oxidative damage can be attributed to their high content of unsaturated fatty acids [31]. Antioxidants, which are essential in protecting cells against oxidative stress, are primarily localized in the cytoplasm [32]. Sperm cells have limited cytoplasmic space and are rich in enzymes that generate free radicals, making them particularly vulnerable to oxidative damage [33]. In this study, the researchers found that 2METE administration led to lipid peroxidation, as evidenced by a significant hike in the testicular MDA concentration. This result is consistent with previous studies by Somade et al. [12] and Somade et al. [13], which reported elevated concentrations of MDA in the testis of rats exposed to 2METE for 21 and 30 days. However, treatment with FERA significantly reduced the testicular concentration of MDA, indicating its antioxidant properties, ability to scavenge free radicals and prevent lipid peroxidation. This finding is consistent with studies by Sacik et al. [34] and Hasanein et al. [35], which demonstrated the protective effect of FERA against testicular oxidative damage induced by torsion-detorsion and lead exposure, respectively.

NO is normally produced by the endothelium and is a major determinant of proper vascular and endothelial function. In inflammation, the production of NO by the vascular system increases significantly and, in association with other free radicals, participates in oxidative stress [36]. This study found that the concentration of NO in the testes was elevated due to 2METE-induced testicular injury, which likely caused tissue inflammation and called for the production of NO in the testes. The study also revealed that FERA was ineffective in preventing the 2METE-induced elevation of NO in the testes. Past studies, including the works of Hasanein et al. [35] and Ahmed and Elmenoufy [37], have reported the NO-lowering effect of FERA in torsion-detorsion and acrylamide-induced testicular toxicity, respectively. However, our findings from this study did not agree with theirs, which may be due to differences in the potency of the toxicants and their primary target or site of the attack.

To alleviate cellular oxidative assault, the testicular cells possess non-enzymatic (GSH) and enzymatic (SOD, CAT, GST, GPx) antioxidants [38]. The first-line defense antioxidants, such as SOD, CAT, and GPx, are known to neutralize superoxide radicals [39], which are readily produced due to the high rate of oxygen consumption in the mitochondria during testicular sperm production [31]. SOD swiftly converts superoxide radicals to hydrogen peroxide, which can cause oxidative damage to cellular components like DNA, proteins, and lipids if allowed to accumulate [40]. Additionally, if hydrogen peroxide accumulates and reacts with iron (Fe^{2+}), it can lead to the formation of the hydroxyl radical ($^{*}OH$) through Fenton's reaction [39]. Therefore, the elimination of hydrogen peroxide by either CAT or GPx is crucial to protect tes-

ticular cells from hydrogen peroxide-induced oxidative damage [41]. Previous studies [12,13] have implicated 2METE in inducing testicular oxidative stress, and the findings from this study corroborate those findings. FERA's intervention improved the testicular antioxidant status (GSH, GST, CAT, SOD, and GPx) by protecting the testicular cells against 2METE-induced production of free radicals that facilitate cellular oxidative stress. The findings from this study agree with the previous studies of Coman and Vodnar [42], who reported the antioxidative stress effect of FERA against streptozotocin-induced diabetes in rats, Mahmoud et al. [43], who reported the prevention of methotrexate-induced liver oxidative stress by FERA in rats, and Valadez-Garcia et al. [44], who reported FERA's therapeutic effect against γ-irradiation-induced oxidative stress in the testes of rats.

Nrf2 is a key transcription factor that is redox-sensitive and serves as a cellular sensor (in vivo) of oxidative stress. Nrf2 becomes activated in cellular oxidative stress and finds its way to the nucleus where it associates with the promoter of the antioxidant response element to induce and activate a variety of anti-oxidative stress genes [45]. In this study, 2METE-induced testicular activation of the Nrf2 gene was recorded. This can be attributed to the 2METE-induced oxidative stress that was marked by a decrease in the antioxidant parameters (CAT, GSH, SOD, GST, and GPx) obtained in this study. We also propose that the 2METE-induced depletion of endogenous antioxidants was sensed in the testis of rats, which adaptively made the testicular cells respond by activating the Nrf2 signaling pathway. This agrees with the outcomes of previous studies conducted by Somade et al. [13] and Somade et al. [15], where exposures of rats to 2METE resulted in Nrf2 gene activation in the testis and liver of rats, respectively. On the other hand, FERA's intervention prevented the testicular activation of the Nrf2 gene. This effect of FERA can be attributed to its ability to shield the testicular cells against 2METE-induced oxidative stress, which ensured the maintenance of normal endogenous antioxidant status and an unwarranted testicular activation of the Nrf2 gene in the rats. This effect of FERA on testicular Nrf2 gene expression had also been similarly demonstrated by syringic acid in the liver and testis of rats administered 2METE [13,15]. Nrf2 guards against cellular stressors such as free radicals, xenobiotics, environmental toxins, and radiation. Thus, the activated Nrf2 pathway can be a very good means for chemoprevention. Foods such as cruciferous vegetables that contain (isothiocyanates) [46], onions and garlic that contain organosulfur compounds [47], spice turmeric, and green tea that contain polyphenols [48], and soybeans that contain isoflavones [49] have all been reported as strong modulators of the Nrf2 pathway. Like FERA, epigallocatechin-3-gallate [50,51], diallyl trisulfide [52], curcumin [53,54], and sulforaphane [55] are some of the compounds documented to possess cell-protecting effects against diabetic neuropathy, cardiovascular disease, neurodegeneration, and carcinogenesis through modulation of the Nrf2 pathway.

Furthermore, some of the anti-oxidative stress genes under the control of Nrf2 are CAT, SOD, GST, Hmox1, and NQO1. Hmox1 is the rate-limiting enzyme in heme degradation and serves as an important mechanism for Nrf2 to demonstrate its antioxidant effect, through the former's ability to degrade heme into biliverdin, carbon monoxide, and iron (II) [56,57]. The NQO1 gene, another downstream target of Nrf2 transcription, can be used to study the Nrf2 pathway [58]. Induction of the NQO1 gene by Nrf2 transcription activity is needed for the former (NQO1) to catalyze the 2-electron reduction of quinones to form less toxic hydroquinones [13]. In this study, the Hmox1 gene was activated while the NQO1 gene was downregulated in the testis of rats administered 2METE only. These contrasting effects of 2METE administrations on the expressions of these Nrf2-controlled genes can be attributed to the induction of oxidative stress in the rats. In addition, the activated cytosolic Nrf2 gene may have relocated into the nucleus in response to the 2METE-induced oxidative stress to induce the gene expressions of NQO1 and Hmox1. For NQO1, the observed decrease in expression may be due to its depletion while exerting its antioxidant role against the 2METE-induced oxidative stress in rats. The inhibition of Hmox1 and upregulation of NQO1 gene expressions by FERA is an indication that it possesses antioxidant and Nrf2-Hmox1-NQO1 signaling pathway modulatory properties that can prevent the 2METE-induced oxidative stress. Again, this regulatory effect of FERA on the testicular Nrf2-ARE pathway had also been similarly demonstrated using syringic acid in the liver and testis of rats administered 2METE [13,15]. Apart from FERA, other plant-derived antioxidants with Hmox1 gene modulatory roles include quercetin [59], curcumin [60], carnosic acid [59], resveratrol [61], celastrol [59], and capsaicin [62].

The forkhead box proteins are a family of transcription regulators that control different cellular processes such as metabolism, resistance to oxidative stress, cell proliferation, apoptosis, and immunity [63]. Of all these cellular processes controlled by FoxO, the one that is of particular interest to this study is oxidative stress. FoxO targets antioxidant proteins that can prevent free radical-induced oxidative damage to biological molecules. Mn-SOD is one of the FoxO-controlled antioxidants [64] that catalyzes the dismutation of superoxide radicals to form hydrogen peroxide. The hydrogen peroxide is further detoxified by CAT, a FoxO3a-controlled antioxidant, into oxygen and water [63]. In this study, the 2METE-induced testicular activation of the FoxO1 gene was recorded. Like Nrf2, the testicular activation or upregulation of the FoxO1 gene may be due to the 2METE-induced testicular oxidative stress in rats, which was characterized by the depletion of endogenous antioxidant proteins. Thus, the activation of the FoxO1 gene in rats administered 2METE only must have occurred as a way of responding to the depleted antioxidants so that more of the enzymes could be produced to combat the testicular oxidative stress. FERA's intervention prevented the testicular activation of the FoxO1 gene, which can be attributed to its antioxidant property against tissue oxidative stress that ensured the conservation of the endogenous antioxidant systems, thereby sparing the testicular activation of the FoxO1 gene in the rats. In a study by Morshneva et al. [65], sodium butyrate (an HDAC inhibitor) was reported to induce the senescence of E1A+Ras transformed cells by down-regulating FoxO and inhibiting free radical accumulation.

The normal functioning of signaling cascades depends on the activity of various receptor ligands, such as cytokines and growth factors, that induce free radicals [66]. Previous studies had shown that tumor suppressor proteins possess antioxidative activity by either expressing or activating antioxidant genes to combat oxidative stress. Tumor suppressor genes regulate many cellular processes, including apoptosis, migration, cell differentiation, cell proliferation, cell cycle arrest, and DNA damage repair [67]. One of these genes is PTEN, which is commonly mutated or deleted in many human cancers. PTEN negatively regulates the PI3K-Akt/PKB pathway by inhibiting the conversion of phosphatidylinositol 4,5-bisphosphate (PIP2) to phosphatidylinositol 3,4,5-triphosphate (PIP3), catalyzed by PI3K. Increased PTEN expression has been shown to modulate the PI3K-Akt/PKB signaling pathway, leading to a reduction in cellular free radical generation [68]. The results of this study also revealed elevated testicular PTEN gene expression in rats exposed to only 2METE. This upregulation of PTEN gene expression may be due to 2METE-induced oxidative stress, which depletes endogenous antioxidants in rats by inducing free radicals. Additionally, the upregulation of the testicular PTEN gene may have occurred to inhibit oxidative stress-induced activation of PI3K that drives the Akt/PKB signaling pathway further. The ability of FERA to inhibit testicular PTEN gene expression can be attributed to its free radical scavenging and antioxidative stress properties that help preserve testicular antioxidant markers and downregulate PTEN gene expression in rats. Similarly, ellagic acid isolated from Sanguisorba officinalis was reported to cause G1 cell cycle arrest via the modulation of PTEN in melanoma (B16F10) cells [69].

5. Conclusion

In this study, administration of 2METE resulted in testicular oxidative stress, characterized by a significant elevation in the concentrations of MDA and NO, a significant reduction in the level of GSH, and a de-

University of Ibadan Library

crease in the activities of GST and SOD, as well as reduced activities of CAT and GPx, which led to the downregulation of NQO1 gene and the activation of Nrf2, Hmox1, FoxO1, and PTEN genes in rats. On the other hand, administration of FERA prevented the testicular oxidative stress by significantly decreasing the testicular level of MDA, restoring the concentration of GSH and activities of SOD, GST, GPx, and CAT, as well as modulating the testicular Nrf2-Hmox1-NQO1 signaling pathway, and downregulating the testicular gene expressions of FoxO1 and PTEN in rats.

Limitations

Due to a lack of funding, the entire research study was funded by the authors. This limited us to using chemical methods to assay for the antioxidant markers instead of purchasing their ELISA kits. Although this made the study more challenging, it enabled us to be more careful, and precise, and gain a better understanding of the assay protocols. Also, the unavailability of funds affected the experimental design (sample size), as we were limited to just 4 groups of rats, and many of the markers we intended to check such as the inflammatory and apoptotic pathways were dropped. Strengths of the study include data reproducibility, reliability, and accuracy in methods used, and to the best of our knowledge, this is the first study reporting the anti-oxidative stress effect of FERA against 2METE-induced testicular toxicity.

Funding

We did not receive any.

Declaration of Competing Interest

The authors do not have any competing interests to declare.

References

[1] A.D. Bui, R. Sharma, R. Henkel, A. Agarwal, Reactive oxygen species impact on sperm DNA and its role in male infertility, Andrologia 50 (8) (2018) e13012.
[2] D.A. Butterfield, B. Halliwell, Oxidative stress, dysfunctional glucose metabolism and Alzheimer disease, Nat. Rev. Neurosci. 20 (2019) 148–160.
[3] A.O. Dhulqarnain, N. Takzaree, G. Hassanzadeh, H. Tooli, M. Malekzadeh, N. Khanmohammadi, M. Yaghobinejad, S. Solhjoo, T. Rastegar, Pentoxifylline improves the survival of spermatogemnic cells via oxidative stress suppression and upregulation of PI3K/AKT pathway in mouse model of testicular torsion-detorsion, Heliyon 7 (4) (2021) e06868.
[4] Z. Yuan, C. Lin, Y. He, B. Tao, M. Chen, J. Zhang, P. Liu, K. Cai, Near-infrared light-triggered nitric-oxide-enhanced photodynamic therapy and low-temperature photothermal therapy for biofilm elimination, ACS Nano 14 (3) (2020) 3546–3562.
[5] H. Nasri, M. Rafieian-Kopaei, Protective effects of herbal antioxidants on diabetic kidney disease, J. Res. Med. Sci. 19 (1) (2014) 82–83.
[6] M. Bahmani, M. Mirhoseini, H. Shirzad, M. Sedighi, N. Shahinfard, M. Rafieian-Kopaei, A review on promising natural agents effective on hyperlipidemia, J. Evid. Based Complementary Altern. Med. 20 (3) (2015) 228–238.
[7] M. Rafieian-Kopaei, Plants antioxidants: from laboratory to clinic, J. Nephropathol. 2 (2) (2013) 152–153.
[8] R.H. Al Omari, M.H. Almatarneh, A.Y. Alnajajrah, M.S. Al-Sheraideh, S.S. Al Abbad, Z.H.A. Alsunaidi, Thermal degradation and bimolecular decomposition of 2-ethoxyethanol in binary ethanol and isobutanol solvent mixtures: a computational mechanistic study, ACS Omega 6 (20) (2021) 13365–13374.
[9] O.T. Somade, B.E. Oyinloye, B.O. Ajiboye, O.A. Osukoya, O.E. Adeyi, Effect of syringic acid on steroid and gonadotropic hormones, hematological indices, sperm characteristics and morphologies, and markers of tissue damage in methyl cellosolve-administered rats, Biochem. Biophys. Rep. 32 (2022) 101360 a.
[10] O.T. Somade, B.O. Ajayi, M.O. Olushola, E.O. Omoseebi, Methyl cellosolve-induced renal oxidative stress and time-dependent up-regulation of pro-inflammatory cytokines, apoptotic, and oncogenic markers in rats, Toxicol. Rep. 7 (2020) 779–787 c.
[11] O.T. Somade, B.O. Ajayi, O.E. Adeyi, A.A. Adeshina, M.O. Adekoya, R.O. Abdulhameed, Oxidative stress-mediated induction of pulmonary oncogenes, inflammatory, and apoptotic markers following time-course exposure to ethylene glycol monomethyl ether in rats, Metab. Open 9 (2021) 100075.
[12] O.T. Somade, B.O. Ajayi, O.E. Adeyi, A.A. Adeshina, A.S. James, P.F. Ayodele, Ethylene glycol monomethyl ether-induced testicular oxidative stress and time-dependent up-regulation of apoptotic, pro-inflammatory, and oncogenic markers in rats, Metab. Open 7 (2020) 100051 a.
[13] O.T. Somade, B.O. Ajiboye, O.A. Osukoya, T.A. Jarikre, B.E. Oyinloye, Syringic acid ameliorates testicular oxidative stress via the conservation of endogenous antioxidant markers and inhibition of the activated Nrf2-Keap1-NQO1-HO1 signaling in methyl cellosolve-administered rats, Pharmacol. Res.: Modern Chin. Med. 6 (2023) 100207 a.
[14] O.T. Somade, B.O. Ajayi, O.E. Olunaike, L.A. Jimoh, Hepatic oxidative stress, up regulation of pro-inflammatory cytokines, apoptotic and oncogenic markers following 2-methoxyethanol administrations in rats, Biochem. Biophys. Rep. 24 (2020) 100806 b.
[15] O.T. Somade, B.E. Oyinloye, B.O. Ajiboye, O.A. Osukoya, Methyl cellosolve-induced hepatic oxidative stress: the modulatory effect of syringic acid on Nrf2-Keap1-Hmox1-NQO1 signaling pathway in rats, Phytomed. Plus 3 (2023) 100434 b.
[16] R.J. Boatman, International industry initiatives to improve the glycol ether health effects knowledge base, Toxicol. Lett. 156 (1) (2005) 39–50.
[17] N.D. Raj, D. Singh, A critical appraisal on ferulic acid: biological profile, biopharmaceutical challenges and nano formulations, Health Sci. Rev. 5 (2022) 100063.
[18] Z. Chen, C. Zhang, F. Gao, Q. Fu, C. Fu, Y. He, J. Zhang, A systematic review on the rhizome of Ligusticum chuanxiong Hort. Chuanxiong, Food Chem. Toxicol. 119 (2018) 309–325.
[19] L. Hu, X. Song, T. Nagai, M. Yamamoto, Y. Dai, L. He, H. Kiyohara, X. Yao, Z. Yao, Chemical profile of Cimicifuga heracleifolia Kom. and immunomodulatory effect of its representative bioavailable component, cimigenoside on Poly(I:c)-induced airway inflammation, J. Ethnopharmacol. 267 (2021) 113615.
[20] M.S. Fekri, H.R. Poursalehi, F. Sharififar, A. Mandegary, F. Rostamzadeh, R. Mahmoodi, The effects of methanolic extract of Glycyrrhiza glabra on the prevention and treatment of bleomycin-induced pulmonary fibrosis in rat: experimental study, Drug Chem. Toxicol. 44 (4) (2021) 365–371.
[21] A. Jiso, P. Khemawoot, P. Tachapichetvanich, S. Soopairin, K. Phoemsap, P. Damrongsakul, S. Wongwiwatthananukit, P. Vivithanaporn, Drug-herb interactions among Thai herbs and anticancer drugs: a scoping review, Pharmaceutical 15 (2) (2022) 146.
[22] S. Chowdhury, S. Ghosh, A.K. Das, P.C. Sil, Ferulic acid protects hyperglycemia-induced kidney damage by regulating oxidative insult, inflammation and autophagy, Front. Pharmacol. 10 (2019) 27.
[23] M. Katerji, M. Filippova, P. Duerksen-Hughes, Approaches and methods to measure oxidative stress in clinical samples: research applications in the cancer field, Oxidat. Med. Cell. Long. 2019 (2019) 1279250.
[24] G.F. Chen, Y. Yuan, H. Jiang, S.Y. Ren, L.X. Ding, L. Ma, T. Wu, J. Lu, H. Wang, Electrochemical reduction of nitrate to ammonia via direct eight-electron transfer using a copper-molecular solid catalyst, Nat. Energy 5 (2020) 605–613.
[25] N. Begum, M.A. Ahanger, L. Zhang, AMF inoculation and phosphorus supplementation alleviates drought induced growth and photosynthetic decline in Nicotiana tobacum by up-regulating antioxidant metabolism and osmolyte accumulation, Environ. Exp. Bot. 176 (2020) 104088.
[26] S. Abdulmalek, A. Eldala, D. Awad, M. Balbaa, Ameliorative effect of curcumin and zinc oxide nanoparticle on multiple mechanisms in obese rats with induced type 2 diabetes, Sci. Rep. 11 (2021) 20677.
[27] O.T. Somade, O.E. Adeyi, B.O. Ajayi, O.O. Asunde, P.D. Iloh, A.A. Adesanya, O.I. Babalola, O.T. Folorunsho, D.A. Olakunle, O.F. Lawal, Syringic and ascorbic acids prevent NDMA-induced pulmonary fibrogenesis, inflammation, apoptosis, and oxidative stress through the regulation of PI3K-Akt/PKB-mTOR-PTEN signaling pathway, Metab. Open 14 (2022) 100179.
[28] H.J. Motulsky, Prism 8 Statistics Guide, GraphPad Software Inc., San Diego CA, 2023 www.graphpad.com.
[29] M. Cheng, J. Zhang, L. Yang, S. Shen, P. Li, S. Yao, H. Qu, J. Li, C. Yao, W. Wei, D. Guo, Recent advances in chemical analysis of licorice (Gan-Cao), Fitoterapia 149 (2021) 104803.
[30] O.T. Somade, O.A. Akinloye, R.N. Ugbaja, M.A. Idowu, Cnidoscolus aconitifolius leaf extract exhibits comparable ameliorative potentials with ascorbate in dimethyl nitrosamine-induced bone marrow clastogenicity and hepatotoxicity, Clin. Nutr. Exp. 29 (2020) 36–48 d.
[31] V. Unsal, M. Cicek, I. Sabancilar, Toxicity of carbon tetrachloride, free radicals and role of antioxidants, Rev. Environ. Health 36 (2) (2021) 279–295.
[32] E. Barati, H. Nikzad, M. Karimian, Oxidative stress and male infertility: current knowledge of pathophysiology and role of antioxidant therapy in disease management, Cell. Mol. Life Sci. 77 (2020) 93–113.
[33] A.V. Snezhkina, A.V. Kudryavtseva, O.L. Kardymon, M.V. Savvateeva, N.V. Melnikova, G.S. Krasnov, A.A. Dmitriev, ROS generation and antioxidant defense systems in normal and malignant cells, Oxid. Med. Cell. Longev. 2019 (2019) 6175804.
[34] U. Sacik, Z. Cavdar, C. Ural, N. Ersoy, C. Ozogul, G. Erbil, Effect of ferulic acid on testicular damage caused by torsion-detorsion in rats, Biotech. Histochem. 98 (2) (2023) 77–85.
[35] P. Hasanein, F. Fazeli, M. Parviz, M. Roghani, Ferulic acid prevents lead-induced testicular oxidative stress and suppressed spermatogenesis in rats, Andrologia 50 (2017) e12798.
[36] H. Wu, Y. Wang, Y. Zhang, F. Xu, J. Chen, L. Duan, T. Zhang, J. Wang, F. Zhang, Breaking the vicious loop between inflammation, oxidative stress and coagulation, a novel anti-thrombus insight of nattokinase by inhibiting LPS-induced inflammation and oxidative stress, Red. Biol. 32 (2020) 101500.
[37] M.M. Ahmed, G. Elmenoufy, Ameliorative effect of ferulic acid on acrylamide-induced inflammation and oxidative damage in rat testes, Res. J. Pharm Biol. Chem. Sci. 7 (1) (2016) 396–403.
[38] H. Wagner, J.W. Cheng, E.Y. Ko, Role of reactive oxygen species in male infertility: an updated review of literature, Arab J. Urol. 16 (1) (2018) 35–43.

University of Ibadan Library

[39] O.M. Ighodaro, O.A. Akinloye, First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): their fundamental role in the entire antioxidant defence grid, Alex. J. Med. 54 (2018) 287–293.
[40] D. Tang, X. Chen, G. Kroemer, Cuproptosis: a copper-triggered modality of mitochondrial cell death, Cell Res. 32 (2022) 417–418.
[41] R. Akhigbe, A. Ajayi, Testicular toxicity following chronic codeine administration is via oxidative DNA damage and up-regulation of NO/TNF-α and caspase 3 activities, PLoS One 15 (3) (2020) e0224052.
[42] V. Coman, D.C. Vodnar, Hydroxycinnamic acids and human health: recent advances, J. Sci. Food Agric. 100 (2) (2020) 483–499.
[43] A.M. Mahmoud, O.E. Hussein, W.G. Hozayen, M. Bin-Jumah, S.M. Abd El-Twab, Ferulic acid prevents oxidative stress, inflammation, and liver injury via upregulation of Nrf2/HO-1 signaling in methotrexate-induced rats, Environ. Sci. Pollut. Res. 27 (2020) 7910–7921.
[44] K.M. Valadez-Garcia, L. Avendano-Reyes, C.A. Meza-Herrera, M. Mellado, R. Diaz–Molina, H. Gonzalez-Rios, U. Macias-Cruz, Ferulic acid in animal feeding: mechanisms of action, productive benefits, and future perspectives in meat production, Food Biosci. 43 (2021) 101247.
[45] J. Li, C. Xiong, P. Xu, Q. Luo, R. Zhang, Puerarin induces apoptosis in prostate cancer cells via inactivation of the Keap1/Nrf2/ARE signaling pathway, Bioengineering 12 (1) (2021) 402–413.
[46] R. Chen, J. Wei, Y. Gao, A review of the study of active components and their pharmacology value in Lepidium meyenii (Maca), Phytother. Res. 35 (12) (2021) 6706–6719.
[47] T.L. Suraweera, H.P.V. Rupasinghe, G. Dellaire, Z. Xu, Regulation of Nrf2/ARE pathway by dietary flavonoids: a friend or foe for cancer management, Antioxidants 9 (10) (2020) 973.
[48] Q. Zhang, J. Liu, H. Duan, R. Li, W. Peng, C. Wu, Activation of Nrf2/HO-1 signaling: an important molecular mechanism of herbal medicine in the treatment of atherosclerosis via the protection of vascular endothelial cells from oxidative stress, J. Adv. Res. 34 (2021) 43–63.
[49] M. Wang, Z. Chen, W. Song, D. Hong, L. Huang, Y. Li, A review on cadmium exposure in the population and intervention strategies against cadmium toxicity, Bull. Environ. Contam. Toxicol. 106 (2021) 65–74.
[50] S. Saha, B. Buttari, E. Panieri, E. Profumo, L. Saso, An overview of Nrf2 signaling pathway and its role in inflammation, Molecules 25 (22) (2020) 5474.
[51] V. Aggarwal, H.S. Tuli, M. Tania, S. Srivastava, E.E. Ritzer, A. Pandey, D. Aggarwal, T.S. Barwal, A. Jain, G. Kaur, K. Sak, M. Varol, A. Bishayee, Molecular mechanisms of action of epigallocatechin gallate in cancer: recent trends and advancement, Semin. Cancer Biol 80 (2022) 256–275.
[52] A. Mondal, S. Banerjee, S. Bose, S. Mazumder, R.A. Haber, M.H. Farzaei, A. Bishayee, Garlic constituents for cancer prevention and therapy: from phytochemistry to novel formulations, Pharm. Res. 175 (2022) 105837.
[53] M.H. Chairez-Ramirez, K.G. de la Cruz-Lopez, A. Garcia-Carranca, Polyphenols as antitumor agents targeting key players in cancer-driving signaling pathways, Front. Pharmacol. 12 (2021) 710304.
[54] G.V.M. Pereira, D.P.C. Neto, A.I.M. Junior, F.G. do Prado, M.G.B. Pagnoncelli, S.G. Karp, C.R. Soccol, Chapter three – Chemical composition and health properties of coffee and coffee by-products, Adv. Food Nutr. Res. 91 (2020) 65–96.
[55] D. Han, X. Gu, J. Gao, Z. Wang, G. Liu, H.W. Barkema, B. Han, Chlorogenic acid promotes the Nrf2/HO-1 anti-oxidative pathway by activating $p21^{Waf1/Cip1}$ to resist dexamethasone-induced apoptosis in osteoblastic cells, Free Rad. Biol. Med. 137 (2019) 1–12.
[56] S.W. Ryter, Heme oxygenase-1: an anti-inflammatory effector in cardiovascular, lung, and related metabolic disorders, Antioxidants 11 (3) (2022) 555.
[57] D. Li, Y. Rui, S. Guo, F. Luan, R. Liu, N. Zeng, Ferulic acid: a review of its pharmacology, pharmacokinetics and derivatives, Life Sci. 284 (2021) 119921.
[58] A. Menzel, H. Samouda, F. Dohet, S. Loap, M.S. Ellulu, T. Bohn, Common and novel markers for measuring inflammation and oxidative stress ex vivo in research and clinical practice – which to use regarding disease outcomes? Antioxidants 10 (3) (2021) 414.
[59] S.C. Funes, M. Rios, A. Fernandez-Fierro, C. Covian, S.M. Bueno, C.A. Riedel, J.P. Mackern-Oberti, A.M. Kalergis, Naturally derived heme-oxygenase 1 inducers and their therapeutic application to immune-mediated diseases, Front. Immunol. 11 (2020) 01467.
[60] N.K. Campbell, H.K. Fitzgerald, A. Dunne, Regulation of inflammation by the antioxidant haem oxygenase 1, Nat. Rev. Immunol. 21 (2021) 411–425.
[61] M. Leri, M. Scuto, M.L. Ontario, V. Calabrese, E.J. Calabrese, M. Bucciantini, M. Stefani, Healthy effects of plant polyphenols: molecular mechanisms, Int. J. Mol. Sci. 21 (4) (2020) 1250.
[62] J. Zhang, D. Duan, Z. Song, T. Liu, Y. Hou, J. Fang, Small molecules regulating reactive oxygen species homeostasis for cancer therapy, Med. Res. Rev. 41 (1) (2020) 342–394.
[63] G. Calissi, E.W.F. Lam, W. Link, Therapeutic strategies targeting FOXO transcription factors, Nat. Rev. Drug Dis. 20 (2021) 21–38.
[64] G. Hoxhaj, B.D. Manning, The PI3K-AKT network at the interface of oncogenic signalling and cancer metabolism, Nat. Rev. Cancer 20 (2020) 74–88.
[65] A. Morshneva, O. Gnedina, S. Svetlikova, V. Pospelov, M. Igotti, Time-dependent-modulation of FoxO activity by HDAC inhibitor in oncogene-transformed E1A+Ras cells, AIMS Genet 5 (1) (2018) 41–52.
[66] S. Arfin, N.K. Jha, S.K. Jha, K.K. Kesari, J. Ruokolainen, S. Roychoudhury, B. Rathi, D. Kumar, Oxidative stress in cancer cell metabolism, Antioxidants 10 (5) (2021) 642.
[67] B. Liu, J. Qu, W. Zhang, J.C.I. Belmonte, G.H. Liu, A stem cell aging framework, from mechanisms to interventions, Cell Rep. 41 (3) (2022) 111451.
[68] I.S. Elgenaidi, J.P. Spiers, Regulation of the phosphoprotein phosphatase 2A system and its modulation during oxidative stress: a potential therapeutic target? Pharmacol Therap 198 (2019) 68–89.
[69] Y.H. Tan, T. Shudo, T. Yoshida, Y. Sugiyama, J.Y. Si, C. Tsukano, Y. Takemoto, A. Kakizuka, Ellagic acid, extracted from Sanguisorba officinalis, induces G1 arrest by modulating PTEN activity in B16F10 melanoma cells, Gen. Cells 24 (2019) 688–704.

University of Ibadan Library