Annals of Clinical Toxicology

Research Article
Published: 26 Feb, 2021

Utility of *Allium cepa* (Onion) Extract in Flubendazole - Testicular Dysfunction

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Abstract

Flubendazole, a common anti-parasitic drug, is clinically used in the treatment of intestinal infections. Recently, reports confirmed its anti-cancer properties. Quercetin on the other hand, has been reported to inhibit the oxidation of other molecules and hence is classified as an antioxidant. This study therefore investigated the possible testicular impairment of Flubendazole and role of apoptosis as well as the ameliorating effect of co-administration of onion extract and quercetin. Groups I, II & III were treated with vehicle (control), 10 mg/kg quercetin and 10 mg/kg onion extract respectively. 5 mg/kg, 10 mg/kg and 20 mg/kg doses of flubendazole were administered to mice in group IV, V and VI respectively. Groups VII and VIII were treated with 20 mg/kg/bwt FB+10 mg/kg quercetin and 20 mg/kg/bwt FB + 10 mg/kg onion extract respectively. FB was administered intraperitoneally and consecutively for 14 days. Results showed that FB caused significant elevation of ROS and MDA levels whereas levels of SOD and GSH were reduced significantly in mice testes. Sperm head count and motility was significantly decreased with simultaneous decrease of sperm morphology by flubendazole treatment at doses of 10 mg/kg/bwt and 20 mg/kg/bwt. Flubendazole also led to compromise in sperm DNA integrity. Increased activities of Caspases 3 and 9 indicate that apoptosis were induced by flubendazole in the rats. However, co-administration with onion extract served to reverse these toxic effects of FB.

Keywords: Flubendazole; Onion extract; Quercetin; Apoptosis; DNA Integrity

Introduction

Flubendazole (FB) is a commonly used anthelmintic drug in clinical practice. FB belongs to a class of aromatic benzimidazoles and is pharmacologically active against a broad range of helminths. As such, it is used in human and veterinary medicine for evacuation of *nematodes* (endoparasitic worms) which live in the gastrointestinal tract [1,2]. FB plays this crucial role by interfering with microtubule formation and glucose uptake by the helminths *via* active binding of tubulin [3,4]. Previous studies have recorded that FB is a good drug for treating tumors [5]. Studies have also revealed that FB is capable of inducing apoptotic cell death in certain cancer cells [6]. Albendazole, an anthelmintic drug in the same class as FB, causes oxidative stress and DNA damage which plays a role in its parasiticidal property [7]. Like FB, albendazole also exhibits cytotoxic effects on endoparasites by binding tubulin. The hallmark of oxidative stress is a heightened production of free radicals which have a negative effect on membrane and DNA integrities [8]. Thus, an enhanced risk of oxidative stress will negatively affect production of important reproductive hormones like testosterone, luteinizing hormone and follicle-stimulating hormone [9]. Furthermore, oxidative stress also plays an indispensable role in apoptosis as reported by previous studies. Studies have revealed that antioxidants prevent of delay apoptosis through their antioxidant functions. Antioxidants from endogenous or exogenous sources are potent to reduce production of ROS at different levels. Examples are quercetin, glutathione, and catalase and superoxide dismutase [10]. Many edible plants are proven to be rich in diverse antioxidants and their benefits are relevant to amelioration of oxidative stress. Onion, *Allium cepa*, is an edible vegetable which has been documented to be a source of dietary quercetin. It is also rich in anthocyanins and a variety of other flavonoids which possess potent antioxidative and antihypertensive properties [11,12]. It is uncertain whether FB also causes oxidative stress as its counterpart albendazole. No records pertaining to this information were found from previous studies. In this study, the effects of different doses of FB on markers of oxidative stress, testicular function, spermatogenic parameters and apoptotic markers were investigated. This work also examined the influence of onion skin extract on parameters aforementioned here.

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Received Date: *06 Feb 2021*
Accepted Date: *23 Feb 2021*
Published Date: *26 Feb 2021*

Citation:
Ojo OO, Ajayi OO, Adewole M. Utility of Allium cepa (Onion) Extract in Flubendazole - Testicular Dysfunction. Ann Clin Toxicol. 2021; 4(1): 1034.

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Materials and Methods

Preparation of onion extracts

Onion bulbs (*Allium cepa L.*) were obtained from a local farm. The onion bulbs were cleaned and air-dried. This process was carried out according to the protocol of Guo-Qing Shi [13]. At 72°C, ethanol (50%) was used to extract 200 g of onion skin powder. The resulting extract was then cooled to room temperature (25°C ± 2°C) and filtered through filter paper. This procedure was done in duplicates.

Animal treatments

All experimental animals were used according to standard protocols prescribed by the Ekiti-State University Animal Ethics Committee (IAEC). Neat cages were used to house the animals while maintaining room temperature and humidity. The animals also had access to standard rat chow and clean water ad libitum. They were housed for a one-week period before induction.

Experimental procedures

40 albino mice (Swiss; weight =25 g) were used in this study. Eight (8) experimental groups were created with each group hosting five (5) rats. The weights of the rats were measured before and after introducing them to flubendazole. After the treatment with flubendazole, the mice were sacrificed and testicular tissues were removed and twice washed with buffer (PBS). The experimental design is shown in Table 1.

Reactive oxygen species (ROS) assay

50 µl of rat homogenate and sodium acetate buffer (1400 µl) were mixed in a cuvette. 1000 µl of standard reagent (6 mg/ml of N,N-diethyl-para-phenylenediamine; 4.37 µM of ferrous sulfate mixed with in sodium acetate buffer (0.1 M, pH 4.8) was introduced to the mixture for five minutes. Absorbance was read using a spectrophotometer (at 505 nm wavelength). Calculation for tissue ROS levels was done using H_2O_2 calibration. The values obtained were expressed as U/mg protein.

Malondialdehyde (MDA) assay

1 g of tissue (testes) was pulverized in phosphate buffer (0.05 M, pH=7.4) and the concentration of 10% (w/v). To supernatant (150 ml), 10% trichloric acid (300 ml) was introduced and then centrifuged at 1000 g for ten min (4°C). The supernatant was incubated with 0.67% thiobarbituric acid in a test tube for twenty-five min at 100°C. A pink coloration was observed due to the action of thiobarbituric acid on malondialdehyde. Absorbance was measured at 535 nm with the aid of a spectrophotometer. Calculation for MDA levels was done through the coefficient of thiobarbituric acid-malondialdehyde absorption complex with values presented in nmol/g of tissue (wet).

Superoxide dismutase (SOD) and Reduced Glutathione (GSH) assays

The activity of SOD and level of GSH were assayed according to standard procedure [14,15].

Sperm count and morphology

After the experiment, the animals were sacrificed and the epididymis was excised and kept in HBSS poured into a Petri dish at normal temperature. The excision of the epididymis permitted the sperms to flow out. The sperm-containing solutions were centrifuged for three min (1000 rpm). A portion of the supernatant was checked for sperm morphology count (using a hemocytometer) and comet assays. The epididymal sperm count was determined by hemocytometer. In sperm morphology assessment, a portion of the sperm-containing solution (0.5 ml) was mixed with 2% eosin (0.5 ml) and left to stand for an hour so as to ensure proper staining. The resulting solution was smeared on glass slides (2 to 3) drops, air-dried and fixed for three minutes with methanol. For each animal, 200 sperms were assessed for morphological irregularities. This was carried out in an oil immersion medium. The results were classified as either normal, slightly normal and abnormal according to the description of [18].

Motility assessment

For sperm motility assessment, semen were extracted and smeared on a glass slide under a lamella. At 400x magnification using a microscope, the movements of the sperms were assessed. The sperm movements were classified as rapidly progressive, slowly progressive, non-progressive or non-motile. The sperms were counted in several microscopic magnifications and the number of mobile and non-motile sperms was recorded.

Testicular cells preparation

The protocol described by Malkov et al. [19] was employed in the preparation of testicular cells. After sacrificing the animals, the testis was obtained and decapsulated. Its contents were channeled into a separate tube with ice-cold buffer (PBS). The resulting solution was subjected to incubation and shaking. After a series of steps, the seminiferous tubules were collected and washed in buffer (PBS).

Caspase 3 and Caspase 9 activities assay

Colorimetric assay kits were used for determining the activities of Caspases 3 and 9 strictly adhering to the manufacturer's guidelines. The testes were pulverized and cells were isolated. PBS buffer was used to wash the homogenate after centrifugation at 500 g. The resulting solution was further extracted and used for calculation of Caspases 3 and 9 activities.

Spermatogenic cells viability assay

A Neubauer hemocytometer was used to count cell number. Cells considered as viable were detected by their non-reaction with the dye used for staining the cells. Every viable cell possesses a clear cytoplasm while non-viable cells have a blue-colored cytoplasm. To obtain the number of viable cells in the mixture, the total number of cells considered as viable was divided by the total cells which were non-viable multiplied by a factor of 100.

Sperm DNA integrity assay

Acridine orange test is a microscopic-based test which functions to determine the rate of change in fluorescence of acridine orange from green to red. Green color represents native DNA while red color represents denatured DNA. It gives an insight to the DNA denaturation status in a cell. Sperm cell solutions were prepared into smears which were fixed in Carnoy's fixative prior to fixation. The slides were rinsed after a few minutes and washed with water (deionized). The sperms were studied under a fluorescent light microscope for red and green colorations. Each slide analyzed at a time contained 100 sperms [20,21].

Statistical analysis

All results were subjected to one-way analysis of variance (ANOVA) using prism statistical package. All values were expressed as mean ± Standard Error of Mean (SEM). At $p<0.05$, values were statistically regarded as significant.

Results

Effect of FB malondialdehyde levels

Significant raised level of malondialdehyde was measured in dose-dependent pattern ($p<0.5$). The level decrease in the samples co-treated with quercetin and onion extract when compared to Group VI (flubendazole alone) (Figure 1).

Effect of FB on ROS levels

Flubendazole at 20 mg/kg/bwt significantly increase the ROS level compared to control group. When co-treated (20 mg/kg/bwt FB+QUC & 20 mg/kg/bwt FB + onion extract) the level of ROS was significantly decrease (Figure 2).

Effect of FB on anti-oxidant enzymes

5 mg/kg/bwt, 10 mg/kg/bwt and 20 mg/kg/bwt in Group IV, V and VI respectfully significantly ($p<0.05$) decreased the GSH level and SOD activity in a concentration-reliant pattern. Co-treatment with QUC and onion extract alleviates the depleting effect as seen the Figure 3 and 4.

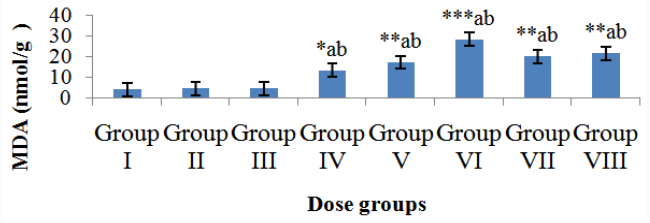

Figure 1: Protective effect of FB+O-E on FB-induced increase in MDA. All the values are expressed as mean ± SEM, (n=5), ***P<0.001, **P<0.01 and *P<0.05, 'a' *vs.* control and 'b' *vs.* FB-20.

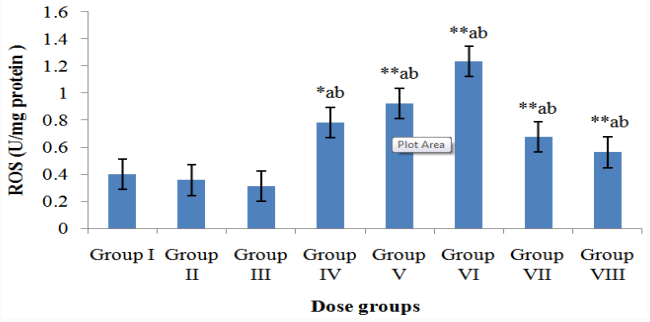

Figure 2: Treatment of FB and co-treatment of FB+O-E on ROS. All the values are expressed as mean ± SEM, (n=5), ***P<0.001, **P<0.01 and *P<0.05, 'a' *vs.* control and 'b' *vs.* FB-20.

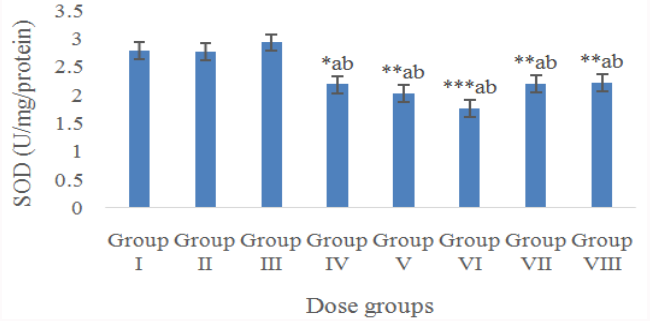

Figure 3: All the values are expressed as mean ± SEM, (n=5), ***P<0.001, **P<0.01 and *P<0.05, 'a' *vs.* control and b *vs.* FB-20.

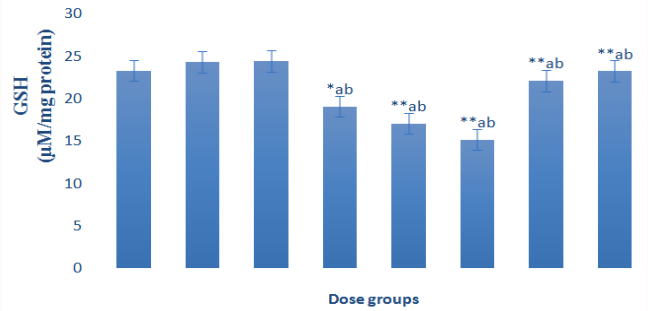

Figure 4: All the values are expressed as mean ± SEM, (n=5), ***P<0.001, **P<0.01 and *P<0.05,' a' *vs.* control and 'b' *vs.* FB-20.

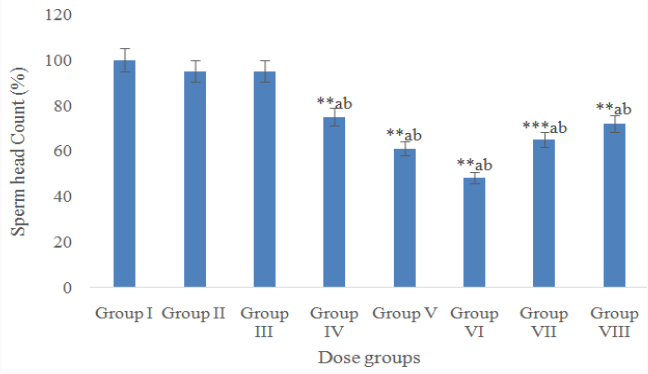

Figure 5: Protective effect of QUC and onion extract against FB on the level of sperm head count. All the values are expressed as mean ± SEM, (n=5), ***P<0.001, **P<0.01 and *P<0.05, 'a' *vs.* control and' b' *vs.* FB-20.

Effect on sperm parameters

In Group VI (20 mg/kg/bwt) significant decrease in the level of sperm head count and sperm motility with simultaneous increase in the level of sperm morphology was measured. Co-treatment of QUC+ 20 mg/kg/bwt FB and onion extract 20 mg/kg/bwt FB decreased sperm head count, sperm morphology and increase sperm motility (Figure 5, 6 and Figure 7).

Effect on caspases 3 and 9

Flubendazole treatment decreased the level of Caspase 3 but increased Caspase 9 level acting as a switch on/off activator of caspases as the FB dosage across the group. The effect was reversed in the group co-treated with quercetin and onion extract (Figure 8).

Effect on cell viability

High concentration of Flubendazole leads to a significant

Table 1: Experimental grouping.

Dose groups	Treatment(mg/kg wgt/day)
Group I	Vehicle-treated control
Group II	10 mg/kg, QUC
Group III	10 mg/kg, Onion extract
Group IV	5 mg/kg, FB
Group V	10 mg/kg, FB
Group VI	20 mg/kg, FB
Group VII	20 mg/kg, FB+10 mg/kg Onion extract
Group VIII	20 mg/kg, FB + 10 mg/kg QUC

Table 2: Effect of QUC and Onion extract on FB induced sperm DNA damage in mice.

Dose groups	Sperm DNA damage (%)	Immature sperm (%)
Group I	13 ± 0.17	7.42 ± 1.26
Group II	15 ± 0.22	6.3 ± 0.12
Group III	16 ± 0.31	9.78 ± 0.21
Group IV	10 ± 0.11^{***ab}	11.52 ± 0.14^{*ab}
Group V	8 ± 0.07^{**ab}	17.77 ± 0.22^{**ab}
Group VI	5 ± 0.27^{***ab}	21.17 ± 0.13^{***ab}
Group VII	11 ± 0.32^{*ab}	12.22 ± 0.22^{*ab}
Group VIII	12 ± 0.12^{*ab}	10.66 ± 0.21^{*ab}

Note: All the values are expressed as mean ± SEM, (n=5), ***P<0.001, **P<0.01 and *P<0.05,' a' *vs.* control and 'b' *vs.* FB-20

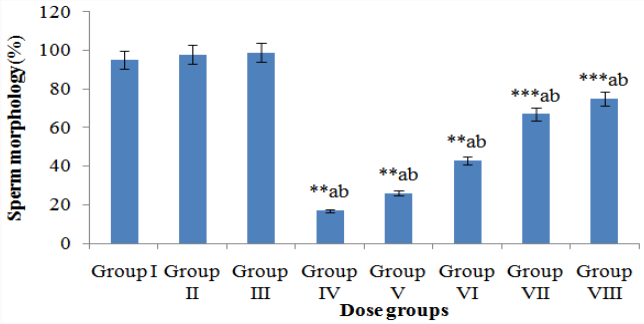

Figure 6: Protective effect of QUC and onion extract against FB on the level of sperm morphology. All the values are expressed as mean ± SEM, (n=5), ***P<0.001, **P<0.01 and *P<0.05, 'a' *vs.* control and 'b' *vs.* FB-20.

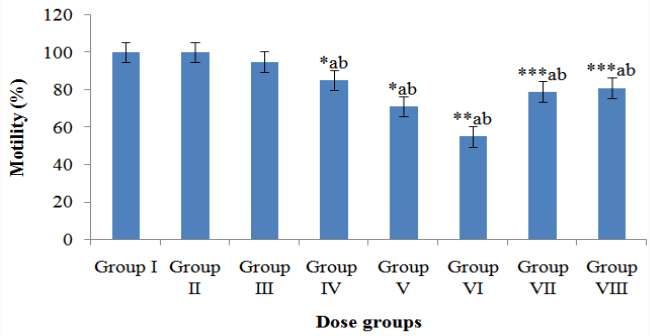

Figure 7: Protective effect of QUC and onion extract against FB on the level of sperm morphology. All the values are expressed as mean ± SEM, (n=5), ***P<0.001, **P<0.01 and *P<0.05, 'a' *vs.* control and 'b' *vs.* FB-20.

decrease in cell viability across the treated group. Co-administration of quercetin and onion extract was able to reverse this effect in a significant manner (Figure 9).

Effect of QUC and onion extract on FB induced sperm DNA damage

High concentration of Flubendazole (20 mg/kg/bwt) treatment lead to a significant increase in both the sperm DNA damage and sperm immaturity compared to the control group while co-treatment with QUC and onion extract (Table 2).

Discussion

The focus of this study was to determine the potential of different doses of Flubendazole (FB) to induce oxidative stress, and also

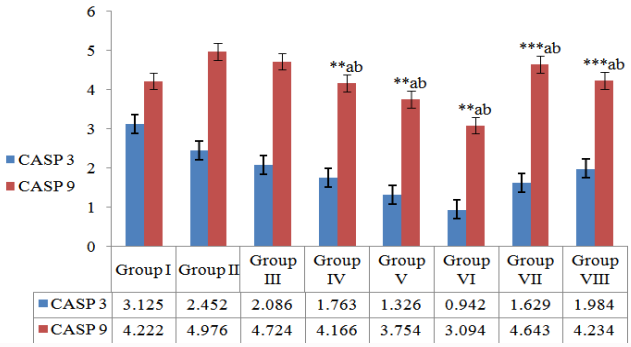

Figure 8: Protective effect of QUC and onion extract against FB on casp-3 and casp-9. All the values are expressed as mean ± SEM, (n=5), ***P<0.001, **P<0.01 and *P<0.05, 'a' *vs.* control and 'b' *vs.* FB-20.

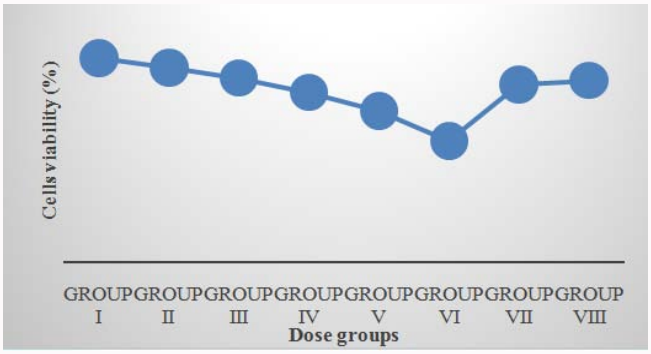

Figure 9: Protective effect of QUC and onion extract against FB on the level of cell viability. All the values are expressed as mean ± SEM, (n=5), ***P<0.001, **P<0.01 and *P<0.05, 'a' *vs.* control and 'b' *vs.* EP-20.

to assess the effects of FB on testicular function, spermatogenic parameters and markers of apoptosis in testicular tissue. From previous research, there is no documentation of the ability of FB to induce oxidative stress in mice especially in testes. From this study, our results reveal that FB can induce oxidative stress in rats. This is a result of the increased levels of Malondialdehyde (MDA) and Reactive Oxygen Species (ROS) observed in mice treated with different doses of FB. ROS are natural but toxic molecular species which are produced as a consequence of aerobic metabolism in normal cells [22,23]. When ROS are produced excessively under certain conditions, they can cause damage to tissues. One of such damages includes irreversible damage to membrane components, which yields MDA in a cellular process called lipid peroxidation [24]. From this study, we propose that increasing concentrations of FB results in increased production of MDA as a result of increased oxidative stress. The highest level of ROS and MDA was produced by the treated mice was observed at 20 mg/kg FB. This may be linked to its metabolism in which oxidative species like superoxide anion, hydrogen radicals and hydrogen peroxide are released [25]. This could also indicate that FB causes depletion of antioxidant defenses [26]. Favorably, ROS and MDA concentrations were significantly lowered ($p<0.05$) on co-administration of FB with onion extract and quercetin. According to previous studies, onion extract has been reported to be rich in quercetin which is a potent antioxidant. This explains its ability to attenuate ROS and MDA levels in testicular tissue. Correspondingly, the levels of SOD and GSH levels in testicular tissue were also improved in testicular tissue of mice treated with onion extract following the levels observed in mice treated with FB.

This supports previous documentation that onion extract is a good source of antioxidant, especially quercetin [11]. Therefore, it may be safe to say that onion extract exhibits antioxidative potential against flubendazole-induced oxidative damage in testicular tissue.

This study also assessed the effects of FB alongside treatment with onion extract on sperm parameters - sperm head count, sperm cell motility & viability, sperm morphology, and sperm DNA integrity. As observed from this study, FB caused a significant reduction ($p<0.05$) in sperm head count, sperm motility, sperm morphology and cell viability in all mice treated with FB. FB treatment also increased production of immature sperm cells and affected sperm cell DNA integrity. Healthy spermatozoa are necessary in male fertility. As such, a compromise in the structure of spermatozoa may lead to male infertility [27]. From the findings collected from this study, FB caused production of reactive oxygen species while depleting antioxidant stores. FB metabolites, especially ROS, may have caused DNA damage in sperm cell mitochondria and nuclei resulting in decreased sperm head count, sperm morphology, motility and cell viability [28,29]. Damaged sperm cell membranes affect ion transport and membrane enzyme activity as well [28]. This could also be responsible for the increased number of immature sperm cells in FB-induced mice as compromise to DNA integrity is characteristic to defective sperm cells and decreased sperm cell viability. However, these effects were reversed in FB-induced mice treated with onion extract. Sperm morphology and head count assays showed that onion extract administered to FB-induced rats improved sperm count and the percentage of sperm cells with normal structure. Cell viability increased while sperm DNA integrity was also restored by onion extract treatment compared to FB-induced mice. The protective effects of onion extract against FB-induced oxidative damage can be attributed to its ability to boost antioxidant defense as observed in the SOD and GSH levels of FB-induced mice treated with onion extract. Improved levels of SOD and GSH protected the testicular tissue against the toxic action of ROS generation stimulated by FB on sperm cell membranes. Reports show that onion contains quercetin as well as other antioxidants like anthocyanins [26]. These antioxidants prevent oxidative damage to sperm cell DNA, maintaining its integrity.

Furthermore, we assessed the effect of FB on caspase 3 and caspase 9 activities in testes of mice testes. Both caspase enzymes are pro-apoptotic in nature. Activation of caspase 9 stimulates a chain of molecular reactions which in turn activates caspase 3 from its zymogen form to signal cell death through the mitochondrial pathway [30]. Apoptosis, programmed cell death, is required for maintaining cellular homeostasis in spermatogenesis so as to prevent overpopulation of cells against the capability of Sertoli cells [31]. Findings from this study revealed decreased activities of both caspase 3 and caspase 9 in FB-induced mice. The lowest activities of caspases 3 and 9 were recorded in the mice that received 20 mg/kg of FB. This indicated that FB impaired apoptosis in the testes. The reason for this can also be traced to the oxidative damage of testicular tissue caused by FB. However, treatment of FB-induced mice with onion extract improved activities of caspase 3 and caspase 9 in testes. This can also be linked with the antioxidative potential exhibited by onion extract. It provides antioxidant defense mechanism against testicular damage.

Conclusively, the current study proposes that FB is able to cause oxidative stress in mice testes. It may also to safe to project that onion extract exhibits attenuative potential against FB-induced testicular dysfunction in male mice.

References

1. Mackenzie CD, Timothy GG. Flubendazole: A candidate macro filaricide for lymphatic filariasis and onchocerciasis field programs. Expert Rev Anti Infect Ther. 2011;9(5):497-501.
2. Kohler P. The biochemical basis of anthelmintic action and resistance. Int J Parasitol. 2001;31(4):336-45.
3. Yadav S, Narasimhan B, Kaur H. Perspectives of benzimidazole derivatives as anticancer agents in the New Era. Anticancer Agents Med Chem. 2016;16(11):1403-25.
4. Hanusova V, Skalova L, Kralova V, Matouskova P. Potential anti-cancer drugs commonly used for other indications. Curr Cancer Drug Targets. 2015;15(1):35-52.
5. Lin S, Yang L, Xu L, Xiang Y, Zhao H, Wang L, et al. Flubendazole demonstrates valid antitumor effects by inhibiting STAT3 and activating autophagy. J Exp Clin Cancer Res. 2019;38(1):293.
6. Tao J, Zhao H, Xie X, Luo M, Gao Z, Sun H, et al. The anthelmintic drug flubendazole induces cell apoptosis and inhibits NF-Kb signaling in esophageal squamous cell carcinoma. Onco Targets Ther. 2019;12:471-8.
7. Martinez-Espinosa R, Arguelo-Garcia R, Saavedra E, Ortega-Pierres G. Albendazole induces oxidative stress and DNA damage in the parasitic protozoan Giadia duodenalis. Front Microbiol. 2015;6:800.
8. Deavall DG, MartinEA, Horner JM, Roberts R. Drug-induced oxidative stress and toxicity. J Toxicol. 2012;2012:645460.
9. Appasamy M, Muttukrishna S, Pizzey A, Ozturk O, Groome N, Serhal P, et al. Relationship between male reproductive hormones, sperm DNA damage and markers of oxidative stress in infertility. Reprod BioMed Online. 2007;14(2):159-65.
10. Kannan K, Jain SK. Oxidative stress and apoptosis. Pathophysiology. 2000;7(3):153-63.
11. Brull V, Burak C, Stoffel-Wagner B, Wolffram S, Nickenig G, Muller C, et al. Effects of a quercetin-rich onion skin extract on 24 h ambulatory blood pressure and endothelial function in overweight-to-obese patients with (pre-)hypertension: A randomized double-blinded placebo-controlled cross-over trial. Br J Nutr. 2015;114(8):1263-77.
12. Cassidy A, Bertoia M, Chiuve S, Flint A, Forman J, Rimm EB. Habitual intake of anthocyanins and risk of cardiovascular disease in men. Am J Clin Nutr. 2016;104(3):587-94.
13. Guo-Qing S, Jing Y, Jiang L, Sheng-Nan L, Han-Xue S, Wen-En Z, et al. Isolation of flavonoids from onion skin and their effects on K562 cell viability. Bangladesh J Pharmacol. 2016;11:S18-25.
14. Marklund S, Marklund G. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur J Biochem. 1974;47(3):469-74.
15. Sedlak J, Lindsay RH. Estimation of total protein-bound and nonprotein sulfhydryl groups in tissue with Ellman's reagent. Anal Biochem. 1968;25(1):192-205.
16. Brown CD, Forman CL, McEuen SF, Miller MG. Metabolism and testicular toxicity of 1,3-dinitrobenzene in rats of different ages. Fundam Appl Toxicol. 1994;23(3):439-46.
17. Ojo OO, Rath SK. Salinomycin Induces Apoptosis In: Germ cells of mouse testis. Eur J Biomed Pharm Sci. 2017;4:9-19.
18. Burruel VR, Yanagimachi R, Whitten WK. Normal mice develop from oocytes injected with spermatozoa with grossly misshapen heads. Biol Reprod.1996;55(3):709–14.
19. Malkov M, Fisher Y, Don J. Developmental schedule of the postnatal rat testis determined by flow cytometry. Biol Reprod. 1998;59(1):84-92.
20. Tayebeh A, Gholamreza N, Vahid N. Protective effect of royal jelly on

fertility and biochemical parameters in bleomycin-induced male rats. Iran J Reprod Med. 2014;12(3):209-16.

21. Varghese A, Fischer-Hammadeh C, Hammadeh M. Acridine orange test for assessment of human sperm DNA integrity. In: Sperm chromatin. Germany: Springer. 2011:189-99.

22. Devasagayan TP, Tilak JC, Boloor KK, Sane KS, Ghaskadbi SS, Lele RD. Free radicals and oxidants in human health; Current status and future prospects. J Assoc Physicians India. 2004;52:794-04.

23. Aglika E. Generation and scavenging of reactive oxygen species in chloroplasts; a submolecular approach. Agric Ecosyst Environ. 2005;106(2):119-33.

24. Grant JJ, Loake GJ. Role of reactive oxygen intermediates and cognate redox signaling in disease resistance. Plant Physiol. 2000;124(1):21-29.

25. Inal ME, Kanbak G, Sunal E. Antioxidant enzyme activities and malondialdehyde levels related to aging. Clin Chim Acta. 2001;305(1-2):75-80.

26. Aktas S, Kum C, Aksoy M. Effects of boric acid feeding on the oxidative stress parameters in testes, sperm parameters and DNA damage in mice. J Trace Elem Med Biol. 2020;58:126447.

27. Aitken RJ, Krausz C. Oxidative stress, DNA damage and the Y chromosome. Reproduction. 2001;122(4):497–506.

28. Tajaddini S, Ebrahimi S, Behnam B, Bakhtiyari M, Joghataei MT, Abbasi M, et al. Antioxidant effect of manganese on the testis structure and sperm parameters of formalin-treated mice. Andrologia. 2014;46(3):246-53.

29. Tedesco I, Carbone V, Spagnuolo C, Minasi P, Russo GL. Identification and quantification of flavonoids from two southern Italian cultivars of Allium cepa L., tropea (red onion) and montoro (copper onion), and their capacity to protect human erythrocytes from oxidative stress. J Agric Food Chem. 2015;63(21):5229–38.

30. Harrington HA, Ho KL, Ghosh S, Tung KC. Construction and analysis of a modular model of caspase activation in apoptosis. Theor Biol Med Model. 2008;5(1):26.

31. Vaithinathan S, D'Cruz SC, Mathur PP. Apoptosis and Male infertility. In: Male infertility: Contemporary clinical approaches, andrology, ART & antioxidants. Germany:Springer. 2012;321-36.