Toxicology International, *Vol 28(1), 57-65, 2021, DOI: 10.18311/ti/2021/v28i1/26296*

Original Research
ISSN (Print): 0971-6580
ISSN (Online): 0976-5131

Protective Effects of Quercetin against Rotenone Induced Histopathological and Biochemical Alteration in Testes of Mice

Juli Jain[1*], Whidul Hasan[1], Rajesh Singh Yadav[2] and Deepali Jat[1]

[1]Neuroscience Research Lab, School of Biological Sciences, Dr. Harisingh Gour Vishwavidyalaya (A Central University), Sagar – 470003, Madhya Pradesh, India; julijain89@gmail.com
[2]Department of Criminology and Forensic Science, Dr. Harisingh Gour Vishwavidyalaya (A Central University), Sagar – 470003, Madhya Pradesh, India

History: Received 2020-10-24; Accepted 2021-01-06; Published 2021-05-24

Abstract

The present work aimed to investigate the protective role of quercetin against rotenone-induced testes damage at the level of oxidative stress and histological alteration. Reproductive toxicity was induced in mice by oral treatment of rotenone, 5 mg/kg body weight daily for 60 days, dissolve in sunflower oil as a vehicle. The quercetin was orally administered 30 mg/kg body weight daily for 60 days along with rotenone. The results of the current study showed that rotenone significantly reduced the Reduced glutathione (GSH) content and decreased the activities of Catalase (CAT) and Superoxide dismutase (SOD) and increased the oxidative markers such as Lipid peroxidation (LPO), and Nitric Oxide (NO) in rotenone treated group as compared to control. Co-administration of quercetin along with rotenone significantly reduced LPO and NO in the testes of mice when compared to rotenone alone group, increased the content of GSH, enhanced activities of CAT, and SOD when compared to rotenone the only group. The results of histological studies of the rotenone treated group showed a reduced number of germ cells, sperms loss, abnormality in seminiferous tubules, and degeneration of interstitial cells was found in the rotenone treated group as compared to control. While co-administration of quercetin reverses these adverse effects. The results of the current study clearly indicated that rotenone exerts harmful effects on the testes and co-administration of quercetin along with rotenone significantly protects damage caused by rotenone.

Keywords: Antioxidant, Oxidative Quercetin, Rotenone, Stress, Testes

1. Introduction

Infertility is defined as the inability to conceive after at least one or two years of unprotected sex. As maximum people can conceive within this time, male infertility is a multi-factorial disease procedure with several possible contributing causes defective in sperm formation is an important reason that contributes to male infertility[1,2]. Experimental studies reveal that the pesticide can interact with the endocrine system and reproductive capacity[3]. The study also has been shown that exposure to pesticides alters the levels of neurotransmitters in agriculture workers[4]. Male sexual dysfunction is composed of numerous problems related to sperm concentration, motility, and hormonal imbalance e.g., low level of testosterone, which is also caused by drug abuse, alcoholism, aging, and cigarette smoking, antidepressant drugs, and exposure to various toxic chemicals[5,6]. Rotenone is a naturally occurring pesticide extracted from the roots of particular plant species involving *Derris mallaccensis, Derris elliptica, Lonchocarpus utilis,*

*Author for correspondence

and *Lonchocarpus urucu* in the family Leguminosae. As it is believed to be a safe natural substitute for synthetic pesticides, rotenone is broadly used as an insecticide in vegetable gardens and to kill or sample fish populations in lakes and reservoirs[7]. Rotenone inhibits mitochondrial complex I (NADH: ubiquinone oxidoreductase), which is a proximal component of the mitochondrial electron transport chain (ETC), and it produces the energy necessary for the maintenance of all neuronal or cell functions. The participation of mitochondrial inhibition in neurodegeneration has received particular attention. Mitochondrial abnormalities have been found in more common sporadic neurological disorders, involving Alzheimer's Disease (AD) and Parkinson's Disease (PD), and they also happen as part of common aging[8]. The chronic exposure of rotenone induces dopaminergic neuronal degeneration and the formation of alpha-synuclein-positive cytoplasmic inclusions in nigral neurons[9]. Rotenone – induces oxidative stress in a different region of the brain in vitro study and in the liver of mice in vivo study[10]. Rotenone – induced histological changes in the cerebellum of mice and also alter the gait performance of mice[11].

PD is a movement disorder and typically it is associated with the disturbance of the nigrostriatal dopaminergic system. In maximum cases of PD, the mesolimbic dopaminergic system is also distressed, which plays a central role as a modulator of the complex reward procedure that organizes motivated behaviors, involving drinking, feeding, and sex, by the boosting of prominent environmental stimuli. The mesolimbic dopaminergic system is recognized to play an essential facility role in copulation[12]. Gonadal steroids, such as testosterone in the male, take part in the nervous system development. A depletion in circulating testosterone contributes to an age-related enhancement in glial fibrillary acidic protein (GFAP) which could render the brain more vulnerable to neurodegeneration during aging[13,14]. Low serum testosterone has been linked with the reduced cognitive presentation in elderly men and AD[15]. Numerous in vivo studies have revealed that androgens can rescue specific populations of motorneurons from both ontogenic- and axotomy-induced death. In in vitro studies, it has been revealed that glucose-deprived hippocampal neurons are protected by testosterone, and testosterone itself also acts as an antioxidant via androgen- receptor-mediated mechanism[16]. The impact of testosterone in neurodegenerative diseases, like AD and PD, can be decoded into new cures of the disorders with distinct sex-specific and more effective therapies. In contrast, gonad steroids enhance the number and size of tyrosine hydroxylase (TH) immunoreactive neurons in a few dopaminergic neuron populations in both male and female rats[17,18]. Thus, it appears that androgen levels are capable of inducing dopaminergic function by modulation of mRNA expression of TH. The sex hormone testosterone has a common and more noticeable impact on the brain than reproduction and sexuality. Alam and Schmidt (2004) reported that the treatment of rats with rotenone (2 mg/kg i.p.) for 60 days decreased testosterone levels in the peripheral blood plasma[19] suggesting that low levels of testosterone due to rotenone maybe contribute to brain-related disorders.

Quercetin, a flavonoid, present in various types of vegetables and fruits[20], has been documented as a strong antioxidant, which is capable of diminishing oxidative stress and cell death by causing chelation of metal ions, reduction of oxygen free radicals and offers defense against lipid peroxidation[21]. In another study, quercetin protects etoposide-induced toxicity in male rats testicular[22]. Quercetin has been documented to protect testicular cells from oxidative stress induced by environmental chemicals like arsenic[23], bisphenol A[24]. From the above statement, we can understand that degeneration in cells that are responsible for the secretion of testosterone leads to a low level of testosterone which may further contribute to increasing the brain vulnerability to neurodegenerative disorders. Although it is well known that maximum pesticides have a marked influence on the reproductive organ, most of them are however not complex I inhibitors. To date, scientific literature carries little evidence regarding the effects of rotenone on the reproductive organ of the male. Therefore, the present study aims to discover the toxicity of rotenone on mice testes at the oxidative stress level and histological level and whether its toxicity is protected by quercetin or not. This study may also contribute to the treatment of neurodegenerative disorders caused by reproductive toxicity.

2. Material and Methods

2.1 Chemicals

Hydrochloric acid (HCl), Trichloroacetic acid (TCA), 5,5'-dithiobis 2-nitrobenzoic acid (DTNB), di-nitrophenylhydrazine (DNPH), ethanol, sodium hydroxide (NaOH), and quercetin were purchased from HiMedia Laboratories (Mumbai, India). Sodium chloride and ethylene diamine tetraacetate (EDTA) and potassium dihydrogen orthophosphate (KH2PO4), were purchased from LOBA, Chemie, India. Rotenone was purchased from MP Biomedicals, France.

2.2 Experimental Animals and Treatment

Adult male *Swiss albino* mice, 3-4 months old weighing (25-30 g) were purchased from the College of Veterinary

Science and Animal Husbandry Mhow, India, and acclimatized for 2 weeks before starting the experiment. Mice were housed in clean plastic cages under standard hygiene conditions, at the temperature 24-28°C with a 12-hour light/dark cycle. Mice were allowed to take standard food and water ad libitum. The mice were divided into three groups having 6 mice in each group. In group I, mice were treated with vehicle and served as the control, in group II, mice were treated with rotenone (5 mg/kg, b.w., p.o. dissolved in sunflower oil) for 60 days, group III were co-treated with quercetin (30 mg/kg, b.w., p.o. dissolved in sunflower oil) and rotenone for 60 days. For the biochemical study, after the last day of treatment, four mice from all the experimental groups were sacrificed by decapitation. Testes tissue was weighed and minced into small pieces with scissors and homogenized in (10% w/v) homogenate buffer PH 7.4, using Teflon homogenizer (Remi Stirrer, 125S/U, Bombay India). Homogenate was centrifuged (1000 rpm for 10 min at 4 °C) to remove the debris and the supernatant was taken out. The supernatant was again centrifuged (12,500 rpm for 15 min at 4°C), and the pellet was discarded. The clear supernatant fluid was taken for analysis of LPO, NO, SOD, CAT, and GSH assays. All procedures were done in accordance with Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment, and the study was approved by the Institutional Animal Ethical Committee (IAEC No. 379/GO/ReBi/S/01/CPCSEA) College of Pharmacy, Sagar (MP), India.

2.3 Histopathological Study

After the last day of treatment, two mice from each treatment group were perfused transcardially with ice-cold 0.1 M phosphate-buffered saline (PBS) PH-7.4, followed by cold paraformaldehyde (4%, w/v) in 0.1 M PBS. After that, the testes were isolated and processed for paraffin-embedded sectioning. Six-micron sections were cut with a microtome, mounted on clean gelatin-coated slides, and stained by hematoxylin and eosin, and observed under a light microscope.

2.4 Biochemical Analysis

2.4.1 Determination of LPO

LPO was estimated by `measuring the MDA amounts by the use of the thiobarbituric acid (TBA) color reaction at a temperature of 95°C for 30 min to form a TBA-reactive product and then resultant pink-colored pigment trimethylene complex product was measured maximum absorbance at 532 nm using spectrophotometer by the method of Ohkawa *et al.*,[25]. The results were expressed as nmol MDA/mg protein.

2.4.2 Determination of GSH

The content of GSH was estimated by the procedure of Hasan[26]. DTNB (5,5'-dithiobis (2-nitrobenzoic acid) or Ellman´s reagent is reduced by the free sulfhydryl group on GSH molecule to yield generate 5-to-2-nitrobenzoic acid which has a yellow color and can be determined by reading absorbance at 412 nm and expressed as umol/mg protein.

2.4.3 Determination of NO Levels

NO is produced from arginine by Nitric oxide synthase (NOS). Nitrate is converted to nitrite by nitrate reductase. The level of NO was determined by measuring the accumulation of nitrite using Griess reagent. Nitrite ions react with Griess reagent to form a pink diazo dye by diazonium coupling reaction which is measured at 540 nm as described earlier by Miranda[27] and expressed as µMol/mg protein.

2.4.4 Determination of SOD

The SOD activity was determined by spectrophotometrically at 560 nm using the modified method of Kakkar[28]. Briefly, the assay mixture containing sodium pyrophosphate buffer (pH 8.3, 0.052M), nitro blue tetrazolium (300 uM), phenazine methosulfate (186 uM), NADH (780 uM), and appropriately diluted enzyme in the total volume of 3mL was kept at 37°C for 90 s. The reaction was stopped by adding glacial acetic acid. The reaction mixture was mixed vigorously by adding n-butanol and was allowed to stand for 10 min before the collection of the butanol layer. The intensity of the chromogen in butanol was measured at 560 nm. The SOD activity was calculated in the inhibition rate %.

2.4.5 Determination of CAT

CAT was measured by the method as described by[29], spectrophotometrically in a post-mitochondrial fraction using H2O2 as a substrate. The activity of the catalase was expressed in mmole/min/mg protein.

2.5 Protein Estimation

Protein concentration was estimated by Folin-phenol reaction as described by Lowry *et al.* using BSA as a standard[30].

2.6 Statistical Analysis

All the data were expressed as mean ± SD of the number of animals in each group (n=6). The statistical significance was evaluated by one-way analysis of variance (ANOVA) using Sigma plot version 12.0. Values were considered statistically significant when $p < 0.05$.

3. Results

3.1 Effect on LPO

The results of LPO revealed that the LPO level was significantly increased in the testes (1.97 folds, $p < 0.001$), in rotenone treated mice as compared to control Figure 1. Co-treatment with quercetin along with rotenone significantly decreased the LPO level in the testes (3.29 folds, $p < 0.001$) as compared to the rotenone alone group.

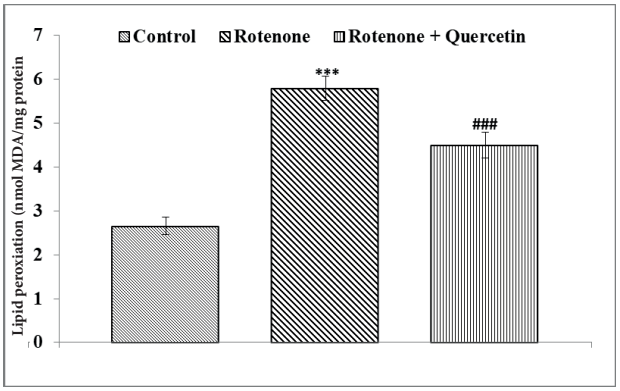


Figure 1. Effect of Quercetin on LPO in mice testes exposed to rotenone . The results were expressed as mean±SE (n=04). *** ($p < 0.001$) Significantly differs from control group, Significantly differs from rotenone treated group (### $p < 0.001$).

3.2 Effect on NO

NO level was increased significantly NO (1.2 folds, $p < 0.001$) in the testes in the rotenone treated group as compared to control Figure 2. Co-treatment with quercetin along with rotenone decreased the level of NO (1.32 folds, $p < 0.001$) in testes as compared to the rotenone alone group.

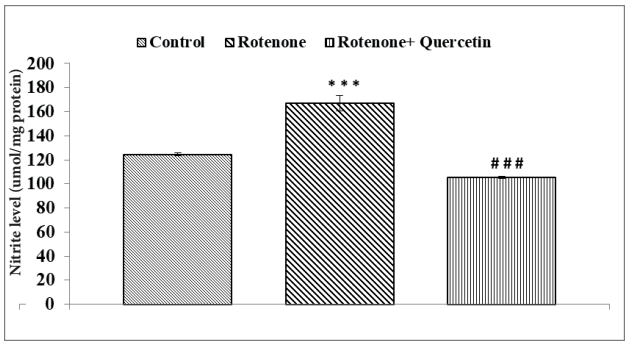


Figure 2. Effect of Quercetin on Nitrite in mice testes exposed to rotenone. The results were expressed as mean±SE (n=04). *** ($p < 0.001$) Significantly differs from control group, Significantly differs from rotenone treated group (# # # $p < 0.001$).

3.3 Effect on GSH content

GSH content was significantly decreased (2.72 folds, $p < 0.001$) in the testes of the rotenone treated group as compared to control Figure 3. Co-treatment with quercetin along with rotenone increased the level of GSH (1.45 folds, $p < 0.01$) as compared to the rotenone alone group.

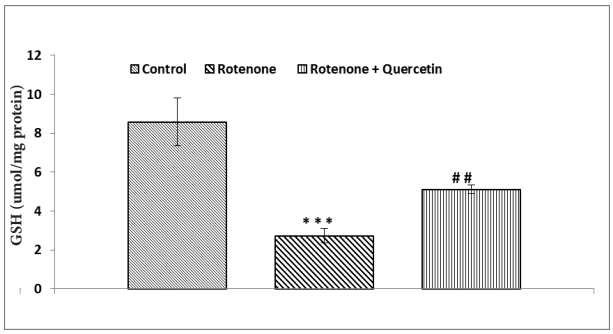


Figure 3. Effect of Quercetin on GSH in mice testes exposed to rotenone. The results were expressed as mean±SE (n=04). The results were expressed as mean±SE (n=04). *** ($p < 0.001$).

3.4 Effect on CAT

The activity of catalase was significantly decreased (3.67 folds, $p < 0.001$) in the rotenone treated group as compared to control Figure 4. Co-treatment with quercetin along with rotenone significantly increased the activity of catalase (1.56 folds, $p < 0.01$) as compared to the rotenone alone group.

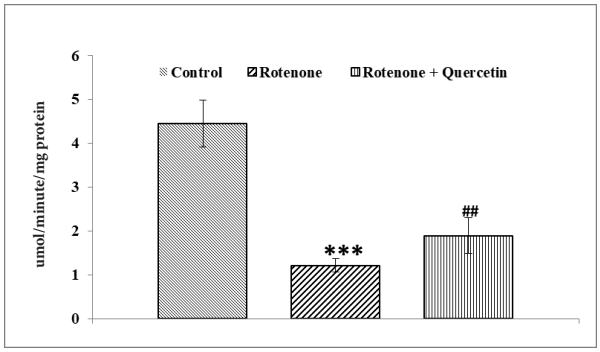


Figure 4. Effect of quercetin on catalase activity in testes of mice following exposure of rotenone. The results were expressed as mean±SE (n=04). *** ($p < 0.001$) Significantly differs from control group, Significantly differs from rotenone treated group (# # $p < 0.01$).

3.5 Effect on SOD activity

The activity of SOD was significantly decreased (1.08 folds, $p < 0.05$) in the rotenone treated group as compared to the control Figure 5. Co-treatment with quercetin along with rotenone significantly increased the activity of SOD (1.05 folds, $p < 0.01$) in testes as compared to the rotenone alone group.

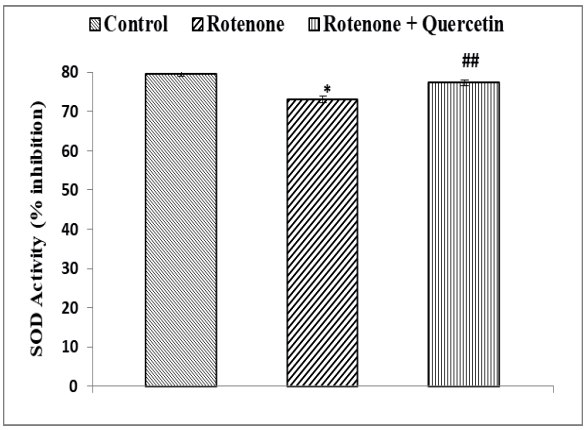


Figure 5. Effect of quercetin on catalase activity in testes of mice following exposure of rotenone. The results were expressed as mean±SE (n=04). *** ($p < 0.001$) Significantly differs from control group, Significantly differs from rotenone treated group (# # $p < 0.01$).

3.6 Histological Results

The present study showed that chronic exposure to rotenone treatment to mice produced oxidative stress as shown by increased levels of LPO and NO and reduced GSH content, decreased activities of CAT and SOD, this oxidative stress leads to histological alteration in testes. Testicular tissue of the control group mice contained normal seminiferous tubules in which spermatogenesis stages are maintained (Figure 6). The germinal epithelium is well arranged and the interstitial tissue seemed with a normal structure having blood vessels. On the other hand, mice treated with rotenone showing degenerative changes (red star) in spermatogenic cells; the detachment of the germinal spermatogenic epithelium (blue arrows with two heads); damaged interstitial tissue (black arrows) and altered spermatogenesis (green star) (Figure 6A). While co-administration of quercetin showing recovery of the spermatogenic epithelium in seminiferous tubules and normal spermatogenesis (Figure 6). Testes tissue sections were stained with hematoxylin and eosin method (400x).

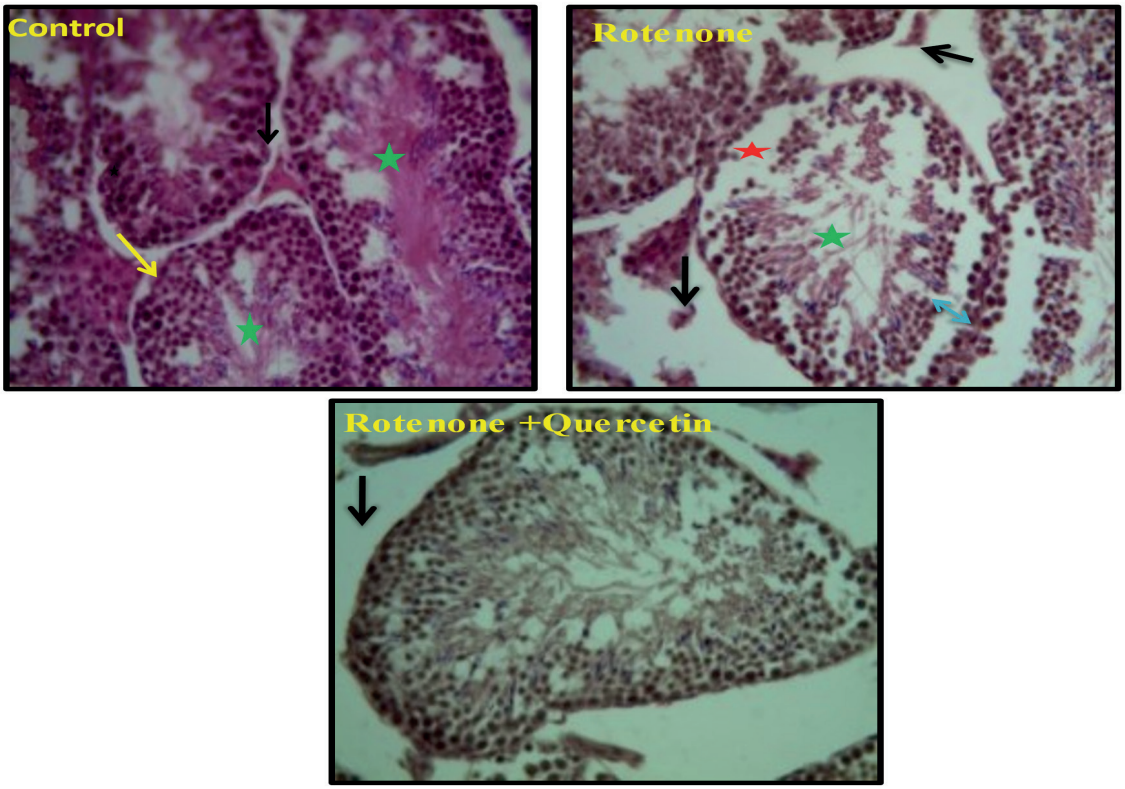


Figure- 6 A Histopathological alterations in testis of mice exposed to rotenone: Photomicrograph of the testicular tissue of the control group mice showing spermatogonia (Yellow arrow) ;interstitial space (black arrow) and active spermatogenesis (green star).
Photomicrograph of the testicular tissue of mice treated with rotenone showing degenerative changed (red star) in spermatogenic cells ; the detachment of the germinal spermatogenic epithelium (blue arrows with two heads);damaged interstitial tissue (black arrows) and altered spermatogenesis (green star).
Photomicrograph of the testicular tissue of mice treated with rotenone + quercetin
showing a recovery of spermatogenic epithelium in seminiferous tubules and normal spermatogenesis.
Testis tissue sections were stained with hematoxylin and eosin method (40x).

3.7 Scanning Electron Microscopy (SEM)

The surface topography and composition was testes analysed through a scanning electron microscope (NOVA NANO SEM-450450 made in Netherland) functional at 15-18 kV. Just before imaging, the experimental samples were coated with gold using a sputter-coater (Denton Desk V) in an inert atmosphere. SEM study of testes sections were done on paraffin sections prepared for histological study. 6 um thick sections of testes were dried at 37°C for overnight and deparaffinised in xylene for 20 min. Then, sections were rinsed with trichloro-trifluoro-ethane (C2Cl3F3) and solvent was seared by evaporation. Then, the backscattered electrons (BSE) were utilized for noticing the different constituents in liver, kidney and testes from surface of dried sections. The surface conductive coating of gold (Au) was used for further investigation. Images were taken at different magnifications i.e. low and high (Boyde and Reid, 1983) through scanning electron microscopy.

3.8 Scanning Electron Microscopy Study of Testes

The SEM study, of testes section shown that degeneration in leading cells and pronounced histological alteration around the Sertoli cells and altered spermatogenesis were found in the lumen of rotenone treated group as compared to control group. While co-administration of quercetin prevents the degeneration of leading cells and active spermatogenesis were observed as compared to rotenone alone group (Figure 6B).

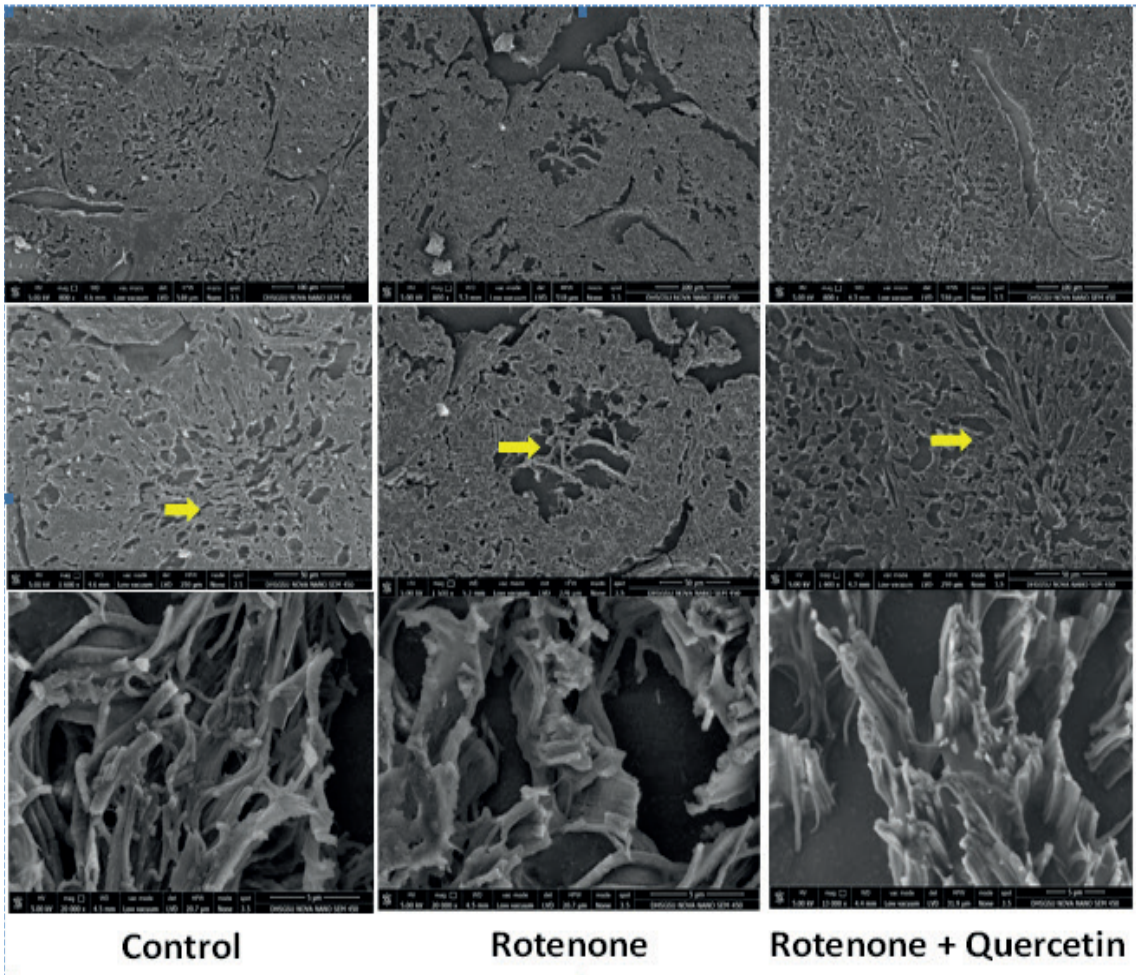


Figure- 6B. Photomicrograph of scanning electron micrograph of testes: Scanning electron micrographs of testicular tissue of the control group mice showing normal structure of interstitial space (black arrow)and active spermatogenesis (Yellow arrow). Photomicrograph of the testes of mice administrated with rotenone showing damaged interstitial tissue (black arrows) and changes in spermatogenesis (yellow arrow). Photomicrograph of the testicular tissue of mice treated with rotenone + quercetin displaying a recovery of spermatogenic epithelium in seminiferous tubules and normal spermatogenesis. Scale Bar 100 µm, 50 um and 5 µm.

4. Discussion

Many studies are available on the rotenone toxicity on the brain but little researches are available on its toxicity on the testes and its suitable medication for neutralizing or reducing its negative side effects has received little attention. In the present study, the protective effect of quercetin on rotenone-induced testes damage was investigated. Data from our study showed that rotenone treatment significantly induced histological changes; reduced CAT and SOD activities and GSH content and enhanced LPO and NO contents in testicular tissue. Co-treatment of quercetin could effectively attenuate these events. The histological outcomes of current findings show that control mice showed normal seminiferous tubules with epithelium consist of two distinct populations of cells, normal spermatogenic cells, called Sertoli cells which support and nourish the developing spermatozoa, in the intertubular space, Leydig cell and connective tissue cells were present. The space between the seminiferous tubule is normal. Rotenone treated mice showed an abnormality in the seminiferous tubule with disturbed spermatogenesis with detached germ cells from the basement membrane and Leydig cell and connective tissue also degenerated. Our study was supported by another study where short-term treatment of rotenone 10 mg/kg b.w. for 7 days intratubular vacuolization in seminiferous tubule and degeneration of spermatogenic cells[31]. Co-treatment of quercetin along with rotenone showed less altered spermatogenesis as compare to the rotenone alone group and reduced numbers of damage seminiferous tubules and Leydig cells. Consistent with other study quercetin protects against histological changes induced by fenitrothion in rats[32]. Testosterone is formed by Leydig cells in the interstitial space of the testes[33]. The primary function of the Leydig cell is the production of testosterone. As this Leydig cell has degenerated, the testosterone level decreased which leads to neurological disorders suggested by various finding[34]. A study has been also documented that a decrease in testosterone level was found in PD patients. Age-related depletion of testosterone may increase the brain's vulnerability to parkinsonian- or Alzheimer's-like neurodegenerative[19]. Impairment in spermatogenesis as revealed by fewer sperm cells present in rotenone treated mice might be the result of Leydig cell degeneration. Because leydig cell degeneration may be reduced the testosterone level in rotenone-treated mice which may be the cause of altered spermatogenesis in our study. These results suggest that the toxicity of rotenone could also affect the brain which leads to neurological disorders. Abnormality in seminiferous tubules is the action of rotenone on the seminiferous tubules or an indirect effect through inhibition of testosterone production. These findings might be considered as the first report regarding the chronic treatment of rotenone effects on mice testicular tissues and its protection through quercetin. This study is supported by another study where short-term rotenone treatment causes a histological alteration in mice testes such as intratubular vacuolization in the seminiferous tubule, the disintegration of spermatogenic cells, and few sperms in the lumen[31]. This study is supported by other studies where rotenone toxicity affects the testes' function. In another study, rotenone reduces testosterone levels at the dose (2.5 mg/kg body weight) was given orally for 50 days[35]. Results obtained in the present study showed that rotenone-induced degenerative effects in the testes of mice. Co-treatment with quercetin along with rotenone demonstrated that recovery of the spermatogenic epithelium in seminiferous tubules and normal spermatogenesis. Co-treatment of quercetin could effectively reverse these adverse effects. These findings suggest that quercetin can effectively improve Sertoli cell functions. Data from the study showed that quercetin protects Sertoli-germ cell co-culture from oxidative damage induced by atrazine[36]. Protective effects of quercetin in Sertoli cells have also been observed in cadmium-induced oxidative damage[37].

Oxidative stress plays a central role in male infertility[38]. The results of the present study reveal that rotenone generated oxidative stress in mice testes as indicated by increased LPO and NO. lipid peroxide is a crucial procedure in numerous pathological condition and is induced by oxidative stress, lipid peroxide is known to be one of the fundamental mechanisms of the cell damage caused by free radicals after being reacted with lipid causing oxidation, which leads to the release of products like malondialdehyde (MDA), Hydrogen Peroxide (H_2O_2) and Hydroxyl radicals[39]. These intermediates may be responsible for abolishes the structure of plasma membranes of sperm and makes it more delicate as a consequence of the breakdown of unsaturated fatty acids and are more sensitive to the ROS, and it is related to with sperm cluster and its non-motility, reduce in the percentage of live sperms and enhanced percentage of dead sperms, morphologically irregular sperms and impairment of spermatogenesis, which causes to infertility[40]. Our results agree with another study, they reported that treatment of rotenone decreased sperm parameters (viability, motility, and sperm count), total antioxidant capacity (TAC), and increased malondialdehyde (MDA) level[41]. Co-administration of quercetin reduces this oxidative marker. Quercetin inhibits the enzyme Xanthine oxidase, which is the key enzyme in the procedure of lipid peroxidation and thus prevents this process and prevents the cell membrane and mitochondria, This enzyme is one of the enzymes that mediate the procedure of producing free radicals (59). Consistent with another finding, quercetin decreased MDA level in adult male Albino rats against reproductive toxicity induced by atrazine[42]. Along with this administration of rotenone

reduced the activities of SOD, CAT, and content of GSH in the crude homogenate of the testes as compared with control. Treatment of rotenone increased oxidative stress and reduces the activities of SOD and CAT in vivo studies[43]. SOD, CAT, glutathione are antioxidant enzymes present in different subcellular fractions of the testes as well as in the epididymal sperm and epididymis[44]. SOD is considered as the first line of defense against the harmful effects of oxyradicals in the cell by catalyzing the dismutation of superoxide anion to hydrogen peroxide and molecular oxygen. Cat, glutathione reductase and glutathione peroxidase protect cells from highly toxic hydrogen peroxide by catalyzing hydrogen peroxide into water and oxygen. The decreased in the GSH level in this study may be due to direct conjugation of GSH with electrophiles species which are formed increasingly by rotenone exposure. In our present study co-administration of quercetin increases the SOD and CAT activities and GSH content because is the ability of quercetin, via two aromatic rings in its structure, can penetrate the phospholipid membranes and upregulation of antioxidant[45]. Our study is consistent with another study where quercetin decreased the MDA level and increased the activities of CAT and SOD in the mice toxicity induced by titanium dioxide nanoparticles[46]. In another study where quercetin reduces No, level and increased GSH content in cerebellum and testes of mice when treated with rotenone and rat model of Ischemia/reperfusion injury of testes[47,48]. Superoxide radicals are harmful to PUFA (polyunsaturated fatty acid) and structural proteins of the plasma membrane and the disposition are saturated by SOD. The decline in SOD favors the increase of superoxide radical, as a consequent inhibit CAT. The reduced CAT activity increases H2O2 production[49].

5. Conclusion

We conclude from the above study that treatment of rotenone induces oxidative stress in mice testes and leads to degeneration of germ cells and leyding cells which may lead to the low level of testosterone which may further result in neurological disorders. Co-administration of quercetin along with rotenone reduces oxidative stress and reverse the histological alteration. This study provides new insight where mice testes are protected by quercetin and it may also help in protecting neurological disorders induced by reproductive toxicity.

Taken together, the use of rotenone as a pesticide principally in vegetable gardens and houses is to be depressed. The results clearly demonstrate that quercetin enhances the antioxidants defense system against rotenone-induced reproductive toxicity and offers an indication that it may have a therapeutic role in free radical-mediated diseases. Our results show that the protective effect of quercetin may be due to both an enhance in the activity of the antioxidant defense system by increased the activities of SOD and CAT and increased the content of GSH and inhibition of LPO and NO production.

6. Acknowledgements

The authors are thankful to the Department of Zoology, Dr. Harisingh Gour University, Sagar, (M.P.) to provide several amenities to complete the research.

7. References

1. Kumar N, Singh AK. Trends of male factor infertility, an important cause of infertility: A review of literature. J. Hum. Reprod. Sci. 2015; 8(4):191–6. https://doi.org/10.4103/0974-1208.170370.PMid:26752853.PMCid:PMC4691969
2. Parlaktas BS, Ozyurt B, Ozyurt H, Tunc AT, Akbas A. Levels of oxidative stress parameters and the protective effects of melatonin in psychosis model rat testis. Asian J. Androl. 2008; 10(2):259–65. https://doi.org/10.1111/j.1745-7262.2008.00338.x. PMid:18097523
3. Diamanti-Kandarakis E, Bourguignon JP, Giudice LC, Hauser R, Prins GS, Soto AM, *et al.* Endocrine-disrupting chemicals: An Endocrine Society scientific statement. Endocr Rev. 2009; 30(4):293–342. https://doi.org/10.1210/er.2009-0002. PMid:19502515 PMCid:PMC2726844
4. Kori RK, Mandrah K, Hasan W, Patel DK, Roy SK, Yadav RS. Identification of markers of depression and neurotoxicity in pesticide exposed agriculture workers. J Biochem Mol Toxicol. 2020. https://doi.org/10.1002/jbt.22477
5. Khan RA. Protective effect of *Launaea procumbens* (L.) on lungs against CCl_4-induced pulmonary damages in rat. BMC Compl Alternative Med. 2012; 12:133. https://doi.org/10.1186/1472-6882-12-133. PMid:22909101. PMCid:PMC3522065
6. Sharma R, Biedenharn KR, Fedor JM, Agarwal A. Lifestyle factors and reproductive health: taking control of your fertility. Reprod. Biol. Endocrinol: RB&E. 2013; 11:66. https://doi.org/10.1186/1477-7827-11-66. PMid:23870423. PMCid:PMC3717046
7. Wood DM, Alsahaf H, Streete P, Dargan PI, Jones AL. Fatality after deliberate ingestion of the pesticide rotenone: A case report. Crit. Care. 2005; 9(3):R280–4. https://doi.org/10.1186/cc3528. PMid:15987402. PMCid:PMC1175899
8. Weidling I, Swerdlow RH. Mitochondrial dysfunction and stress responses in Alzheimer's Disease. Biology.

2019; 8(2). https://doi.org/10.3390/biology8020039. PMid:31083585. PMCid:PMC6627276

9. Zeng XS, Geng WS, Jia JJ. Neurotoxin-induced animal models of parkinson disease: Pathogenic mechanism and assessment. ASN neuro. 2018; 10. https://doi.org/10.1177/1759091418777438. PMid:29809058. PMC id:PMC5977437
10. Hasan W, Kori RK, Thakre K, Yadav RS, Jat D. Synthesis, characterization and efficacy of mitochondrial targeted delivery of TPP-curcumin in rotenone-induced toxicity. Daru J. Pharm. Sci. 2019; 27(2):557–70. https://doi.org/10.1007/s40199-019-00283-2. PMid:31264184. PMCid:PMC6895339
11. Hasan W, Kori RK, Jain J, Yadav RS, Jat D. Neuroprotective effects of mitochondria-targeted curcumin against rotenone-induced oxidative damage in cerebellum of mice. J Biochem Mol Toxicol. 2020; 34(1). https://doi.org/10.1002/jbt.22416
12. Love TM. Oxytocin, motivation and the role of dopamine. Pharmacol Biochem Behav. 2014; 119:49–60. https://doi.org/10.1016/j.pbb.2013.06.011. PMid:23850525. PMCid:PMC3877159
13. Day JR, Frank AT, O'Callaghan JP, Jones BC, Anderson JE. The effect of age and testosterone on the expression of glial fibrillary acidic protein in the rat cerebellum. Exp Neurol. 1998; 151(2):343–6. https://doi.org/10.1006/exnr.1998.6801. PMid:9628769
14. Venkateshappa C, Harish G, Mythri RB, Mahadevan A, Bharath MM, Shankar SK. Increased oxidative damage and decreased antioxidant function in aging human substantia nigra compared to striatum: implications for Parkinson's disease. Neurochem Res. 2012; 37(2):358–69. https://doi.org/10.1007/s11064-011-0619-7. PMid:21971758
15. Wahjoepramono EJ, Asih PR, Aniwiyanti V, Taddei K, Dhaliwal SS, Fuller SJ, *et al.* The Effects of Testosterone Supplementation on Cognitive Functioning in Older Men. CNS Neurol Disord Drug Targets. 2016; 15(3):337–43. https://doi.org/10.2174/1871527315666151110125704. PMid:26553159. PMCid:PMC5078598
16. Goodman Y, Bruce AJ, Cheng B, Mattson MP. Estrogens attenuate and corticosterone exacerbates excitotoxicity, oxidative injury, and amyloid beta-peptide toxicity in hippocampal neurons. J. Neurochem. 1996; 66(5):1836–44. https://doi.org/10.1046/j.1471-4159.1996.66051836.x. PMid:8780008
17. Sanghera MK, Grady S, Smith W, Woodward DJ, Porter JC. Incertohypothalamic A13 dopamine neurons: Effect of gonadal steroids on tyrosine hydroxylase. Neuroendocrinology. 1991; 53(3):268–75. https://doi.org/10.1159/000125728. PMid:1674981
18. Yuri K, Kawata M. Region-specific changes of tyrosine hydroxylase-immunoreactivity by estrogen treatment in female rat hypothalamus. Brain Res. 1994; 645(1–2):278–84. https://doi.org/10.1016/0006-8993(94)91661-6
19. Alam M, Schmidt WJ. Mitochondrial complex I inhibition depletes plasma testosterone in the rotenone model of Parkinson's disease. Physiol Behav. 2004; 83(3):395–400. https://doi.org/10.1016/j.physbeh.2004.08.010. PMid:15581661
20. Panche AN, Diwan AD, Chandra SR. Flavonoids: an overview. J. Nutr. Sci. 2016; 5. https://doi.org/10.1017/jns.2016.41. PMid:28620474. PMCid:PMC5465813
21. Mi Y, Zhang C, Li C, Taneda S, Watanabe G, Suzuki AK, *et al.* Protective effect of quercetin on the reproductive toxicity of 4-nitrophenol in diesel exhaust particles on male embryonic chickens. J. Reprod. Dev. 2010. https://doi.org/10.1262/jrd.09-074N. PMid:20035111
22. Alanbaki AA, Mayali HM, Mayali HK. The protective effect of quercetin and hesperidin on etoposide induced toxicity in male rats testicular. JPSR. 2017; 9:1394–405.
23. Baltaci BB, Uygur R, Caglar V, Aktas C, Aydin M, Ozen OA. Protective effects of quercetin against arsenic-induced testicular damage in rats. Andrologia. 2016; 48(10):1202–13. https://doi.org/10.1111/and.12561. PMid:26992476
24. Jahan S, Ain QU, Ullah H. Therapeutic effects of quercetin against bisphenol a induced testicular damage in male Sprague Dawley rats. Syst biol reprod mec. 2016; 62(2):114–24. https://doi.org/10.3109/19396368.2015.1115139. PMid:26787223
25. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal. Biochem. 1979;95(2):351–8. https://doi.org/10.1016/0003-2697(79)90738-3
26. Hasan M, Haider SS. Acetyl-homocysteine thiolactone protects against some neurotoxic effects of thallium. Neurotoxicology. 1989; 10(2):257–61.
27. Miranda KM, Espey MG, Wink DA. A rapid, simple spectrophotometric method for simultaneous detection of nitrate and nitrite. Nitric oxide: Chem. Biol. 2001; 5(1):62–71. https://doi.org/10.1006/niox.2000.0319. PMid:11178938
28. Kakkar P, Das B, Viswanathan PN. A modified spectrophotometric assay of superoxide dismutase. Indian J biochem bio. 1984; 21(2):130–2.
29. Aebi H. Catalase in vitro. Method enzymol. 1984; 105:121–6. https://doi.org/10.1016/S0076-6879(84)05016-3

30. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 1951; 193(1):265–75. https://doi.org/10.1016/S0021-9258(19)52451-6
31. Radad K, Hassanein K, Moldzio R, Rausch WD. Vascular damage mediates neuronal and non-neuronal pathology following short and long-term rotenone administration in Sprague-Dawley rats. Exp Toxicol Pathol. 2013; 65(1–2):41–7. https://doi.org/10.1016/j.etp.2011.05.008. PMid:21676605
32. Saber TM, Abd El-Aziz RM, Ali HA. Quercetin mitigates fenitrothion-induced testicular toxicity in rats. Andrologia. 2016; 48(5):491–500. https://doi.org/10.1111/and.12467. Mid:26264430
33. Zirkin BR, Papadopoulos V. Leydig cells: formation, function, and regulation. Biol Reprod. 2018; 99(1):101–11. https://doi.org/10.1093/biolre/ioy059. PMid:29566165. PMCid:PMC6044347
34. Nassar GN, Leslie SW. Physiology. Testosterone. 2018.
35. Ahmed HH, Abdel-Rahman M, Ali RS, Abdel Moniem A. Protective effect of Ginkgo biloba extract and pumpkin seed oil against neurotoxicity of rotenone in adult male rats. Res. J. Appl. Sci. 2009; 5(6):622–35.
36. Abarikwu SO, Pant AB, Farombi EO. Dietary antioxidant, quercetin, protects sertoli-germ cell coculture from atrazine-induced oxidative damage. J Biochem Mol Toxicol. 2012; 26(11):477–85. https://doi.org/10.1002/jbt.21449. PMid:23132811
37. Bu T, Mi Y, Zeng W, Zhang C. Protective effect of quercetin on cadmium-induced oxidative toxicity on germ cells in male mice. Anatomical record. 2011; 294(3):520–6.https://doi.org/10.1002/ar.21317.PMid:21337715
38. Dobrakowski M, Kaletka Z, Machon-Grecka A, Kasperczyk S, Horak S, Birkner E, *et al.* The Role of oxidative stress, selected metals, and parameters of the immune system in male fertility. Oxid Med Cell Longev. 2018; 2018. https://doi.org/10.1155/2018/6249536. PMid:30254715. PMCid:PMC6142782
39. Gaschler MM, Stockwell BR. Lipid peroxidation in cell death. Biochem Biophys Res Commun. 2017; 482(3):419–25. https://doi.org/10.1016/j.bbrc.2016.10.086. PMid:28212725. PMCid:PMC5319403
40. Oh SY, Sohn YW, Park JW, Park HJ, Jeon HM, Kim TK, *et al.* Selective cell death of oncogenic Akt-transduced brain cancer cells by etoposide through reactive oxygen species mediated damage. Mol Cancer Ther. 2007; 6(8):2178–87. https://doi.org/10.1158/1535-7163.MCT-07-0111. PMid:17699715
41. Sarbishegi M, Gorgich EAC, Khajavi O. Olive leaves extract improved sperm quality and antioxidant status in the testis of rat exposed to rotenone. Nephrourol Mon. 2017; 9(3). https://doi.org/10.5812/numonthly.47127
42. Aziz RLA, Abdel-Wahab A, El-Ela FIA, Hassan NE-HY, El-Nahass E-S, Ibrahim MA, *et al.* Dose-dependent ameliorative effects of quercetin and l-carnitine against atrazine-induced reproductive toxicity in adult male albino rats. Biomed. Pharmacother. 2018; 102:855–64. https://doi.org/10.1016/j.biopha.2018.03.136. PMid:29710542
43. Hasan W, Kori RK, Jain J, Yadav RS, Jat D. Neuroprotective effects of mitochondria-targeted curcumin against rotenone-induced oxidative damage in cerebellum of mice. J Biochem Mol Toxicol. 2019. https://doi.org/10.1002/jbt.22416
44. O'Flaherty C. The enzymatic antioxidant system of human spermatozoa. Andrology. 2014; 2014. https://doi.org/10.1155/2014/626374
45. van Dijk C, Driessen AJ, Recourt K. The uncoupling efficiency and affinity of flavonoids for vesicles. Biochem Pharmacol. 2000; 60(11):1593–600. https://doi.org/10.1016/S0006-2952(00)00488-3
46. Khorsandi L, Orazizadeh M, Moradi-Gharibvand N, Hemadi M, Mansouri E. Beneficial effects of quercetin on titanium dioxide nanoparticles induced spermato genesis defects in mice. Environ Sci Pollut R. 2017; 24(6):5595–606. https://doi.org/10.1007/s11356-016-8325-2. PMid:28035607
47. Hasan W, Rajak R, Kori RK, Yadav RS, Jat D. Neuroprotective effects of mitochondria-targeted quercetin against rotenone-induced oxidative damage in cerebellum of Mice. Int. J. Nutr. Pharmacol. 2019; 9(4):136. https://doi.org/10.1002/jbt.22416
48. Suzen A, Tekin L, Erdemli ME, Erturk N, Aksungur Z, Aktas S. Protective Effects of Hypericum perforatum and Quercetin in a Rat Model of Ischemia/Reperfusion Injury of Testes. Eur J Pediatr Surg. 2018; 28(1):96–100. https://doi.org/10.1055/s-0037-1604397. PMid:28837999
49. Ma X, Deng D, Chen W. Inhibitors and Activators of SOD, GSH-Px, and CAT. J Enzyme Inhib. 2017; 29:207. https://doi.org/10.5772/65936