Egypt. J. Rad. Sci. Applic. **Vol. 33,** No.1, pp. 45-50 (2020)

Egyptian Journal of Radiation Sciences and Applications
http://ejrsa.journals.ekb.eg/

EGYPTIAN ATOMIC ENERGY AUTHORITY

Tadalafil Attenuates γ-Rays-Induced Hepatic Injury in Rats

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TADALAFIL is widely used in the treatment of many disease disorders. Tadalafil is studied for its protective effect against γ-rays-induced hepatic injury in rats. Rats were exposed to 6 Gy-γ-rays then, treated with Tadalafil for 7-days and compared to the control, Tadalafil alone and irradiated groups. Oxidative-stress markers; malondialdehyde (MAD) and myeloperoxidase (MPO) and the antioxidant markers; reduced glutathione (GSH) content, and the relative enzyme activity of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) were evaluated in liver tissues. The anti-inflammatory markers; tumour necrosis factor-α (TNF-α) and interleukin-6 (IL-6) were evaluated in the blood serum. Data revealed a significant reduction in the γ-rays-induced lipid-peroxidation; MDA content, MPO activity, the inflammatory markers levels of TNF-α and IL-6, and an augmentation in the antioxidant marker levels of GSH, GPx, CAT and SOD. It could be concluded that Tadalafil could alleviate the toxic effects of ionizing radiation in liver via the strong anti-oxidant and anti-inflammatory impact, thus could be useful as a radio protective agent in the radiotherapy schedule. The radio protective influence of Tadalafil could need to be explored for further organs in imminent works.

Keywords: γ-rays, Liver, Radio protector, Rats, Tadalafil.

Introduction

Ionized-radiations cause scared adverse dangers through several organ dysfunctions (Sayed et al., 2018). The liver is a radiosensitive tissue that is more prone to radiation injury. In liver cancer patients, the radiotherapy may lead to liver injury which could be a life-limiting factor (Radwan & Hasan, 2019). Ionizing radiation has cytotoxic and cytogenetic properties initiated primarily by the oxidative impairment induced by free radical release (Ma et al., 2019). Acute exposure to whole body γ-irradiation (6-8 Gy) induces hepatotoxicity and inflammation in rats (Ali et al., 2020; Ghorbani et al., 2020). The need for the radio protectives is emergent to protect normal tissues during radiotherapy (Abou-Zeid et al., 2018; Mahgoub et al., 2020).

Tadalafil is a selective phosphodiesterase-5 inhibitor (PDE-5). It is widely used in the treatment of erectile impotence and pulmoarterial hypertension and may also offer clinical benefit in a range of malignancies. It has also a potent immunomodulatory activity that was clinically approved (Pantziarka et al., 2018). It was also used as a medical therapy for portal hypertension for liver cirrhosis, and it may have long-term beneficial effects in compensated cirrhosis cases (Deibert et al., 2018). Tadalafil induces cyto-protection and promotes cardio protection in mice (Koka et al., 2020). Recently, it is prescribed for treating pulmonary hypertension (Shen et al., 2020), and as a novel therapeutic method for the treatment of Alzheimer's disease, lower urinary tract signs as a consequence of non-malignant prostate hyperplasia and in irritable

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Received 29/7/ 2020 ; Accepted 6/9/ 2020
DOI: 10.21608/ejrsa.2020.37607.1102

bowel syndrome besides the related behavior deviations (Sedky & Magdy, 2020; Singh et al., 2020; Zuccarello et al., 2020).

In the exploration for the best active radio protective agent, aspects such as toxicity, number of organs protected, ease of intake, longstanding stability, and attuned with added drugs need to be evaluated (Gandhi et al., 2019; Musa et al., 2020). Thus, in the present study, the authors investigated the radio protective effect of a chemical radio protector, Tadalafil in rats subjected to whole body γ-rays at 6 Gy through oxidant, antioxidant, and inflammatory indices.

Materials and Methods

Animals

40 adult male Swiss albino rats (120-130 g) were obtained from the Egyptian Organization for Biological Product and Vaccines Giza, Egypt. Animals have received normal nutrition and water *ad libitum* and were kept under regular conditions of ventilation, humidity, temperature (20-24°C), and 12-h light-dark phase. Animals were deprived of food, but not water, overnight before samples collection. All experiments were performed in accordance with the animal ethics and the guide for the care and use of laboratory animals (NRC, 2011).

Radiation processing

Animals were placed in a specially designed well-ventilated acrylic container and the whole body of the animals were exposed to 6 Gy, given at a dose rate of 0.40Gy/min using a ^{137}Cs, Gamma-Cell-40 source (Atomic Energy of Canada Ltd, Ottawa, Ontario, Canada) belonging to the NCRRT, Cairo, Egypt.

Chemicals

Tadalafil was purchased from Sigma-Aldrich, St. Louis, USA. All other chemicals and solvents used were of the highest purity grade available.

Experimental design

Animal grouping

Forty rats were divided into four groups (n= 10). Control rat group was administered normal saline (2 ml/ rat) orally using the gastric tube for 7 days as a vehicle. Tadalafil group (each rat was gavage 6 mg Tadalafil/kg body weight orally, suspended in 2 ml normal saline, once daily for 7 days) according to Wang et al. (2017). γ-rays group; animals were subjected to a single dose of the whole body γ-rays (6 Gy) then, received the vehicle with the same dose and period, and Tadalafil & γ-rays group; rats were exposed to γ-rays (6 Gy) then one hour later, rats received Tadalafil with the same dose and period. The animals were fasted overnight then, decapitated in the morning.

Blood and liver samples were collected from rats of different groups under standard laboratory conditions. Biochemical kits (Abcam, UK) were used to measure myeloperoxidase activity (MPO, Catalogue Number: ab105136) in the hepatic homogenates according to the manufacturer's instructions. The absorbance was read at 520 nm. Estimation of malondialdehyde (MDA, Catalogue Number: ZB-MDA-48A) and reduced glutathione (GSH, Catalogue Number: ZB-GSH-96A) levels, and the relative enzyme activity of superoxide dismutase (SOD, Catalogue Number: ZB-SOD-48A), catalase (CAT, Catalogue Number: ZB-CAT-48A) and glutathione peroxidase (GPx, Catalogue Number: ZB-GPX-A48) in the hepatic homogenates were measured using commercial kits (Zellbio GmbH, Germany) according to the manufacturer's instructions. The absorbance was read at 420 nm, 412 nm, 535 nm, 405 nm and 412 nm, respectively. Detection of serum tumor necrosis factor-α (TNF-α, Catalogue Number: MBS2507393) and interleukin-6 (IL-6, Catalogue Number: MBS355410) were performed utilizing ELISA technique (MyBioSource, San Diego, California, USA) according to the manufacturer's instructions. Each sample assay was repeated three times, using the microplate reader (Thermo Scientiic Multiskan MK3, USA).

Statistical analysis

Data were analysed using one-way analysis of variance (ANOVA) followed by LSD post hoc test. The results obtained were expressed by mean ± standard deviation. Differences were considered significant at $P \leq 0.05$ (Snedecor & Cochran, 1994).

Results

As presented in Tables 1-3, the animal group treated with Tadalafil showed non-significant changes in the levels of all estimated biochemical parameters of the current study.

TABLE 1. The markers of hepatic oxidative stress, MDA levels and MPO activities in the liver of different animal groups.

Groups	Control	Tadalafil	γ-rays	Tadalafil & γ-rays
MDA (nmol/mg tissue)	38.34±0.27	37.11±3.91	68.12±7.22[a]	51.14±4.81[b]
MPO (U/mg tissue)	4.65±0.36	4.41±0.46	15.64±1.13[a]	7.32±0.85[b]

[a] Significant difference from the control group
[b] Significant difference from the γ-rays group

TABLE 2. The inflammatory marker levels of TNF-α and IL-6 in the serum of different animal groups.

Groups	Control	Tadalafil	γ-rays	Tadalafil & γ-rays
TNF-α (pg mL)	28.12±2.63	28.65±3.02	53.14±4.15[a]	36.44±4.02[b]
IL-6 (pg/mL)	15.94±1.62	15.02±1.72	33.17±3.15[a]	22.64±2.51[b]

[a] Significant difference from the control group
[b] Significant difference from the γ-rays group

TABLE 3. The antioxidant system capacity marker levels of GSH, GPx, CAT and SOD in the liver of different animal groups.

Groups	Control	Tadalafil	γ-rays	Tadalafil & γ-rays
GSH (U/g tissue)	83.13±9.14	84.43±8.18	51.28±6.14[a]	70.10±7.15[b]
GPx (nmol/g tissue)	29.47±3.17	30.22±3.15	14.16±2.07[a]	23.14±3.11[b]
CAT (U/g tissue)	3.82±0.35	3.81±0.44	2.16±0.27[a]	3.24±0.43[b]
SOD (U/g tissue)	275.14±19.14	256.54±11.12	135.14±14.16[a]	221.01±19.01[b]

[a] Significant difference from the control group
[b] Significant difference from the γ-rays group

As for the markers of liver injury and oxidative damage, there was a significant rise in MDA level and MPO activity in the irradiated rat group as compared to the controls. Treatment with Tadalafil at a dose of 6 mg/kg exerted a significant reduction on these markers (MDA and MPO) when compared to the irradiated group at a dose of 6 Gy γ-rays as shown in Table 1.

The data on serum inflammatory markers and hepatic antioxidant system capacity markers of different animal groups are summarized in Tables (2 & 3) and showed a significant increase in serum level of TNF-α and IL-6 in the irradiated group as compared to the controls. However, marked decreases in hepatic GSH, GPx, CAT and SOD levels was observed in the irradiated group as compared to the controls (Tables 2, 3). These changes were significantly reversed in the rats treated with the Tadalafil when compared to the corresponding irradiated group values (Tables 2, 3).

Discussion

Ionizing radiation is one of the environmental factors that may contribute to liver dysfunction by a mechanism involving oxidative stress (Abd-Al-Haleem et al., 2019). Oxidative stress is an important aspect involved in the mechanism of radiation-induced tissue injury including radiotherapy-mediated complications, and antioxidants which are important concerns for the development of preventive therapeutics for patients (Ghorbani et al., 2020). Therefore, there is a crucial need for therapeutic measures after

the radiation episode. It is already known that PDE-5, including Tadalafil represents a potential approach against the evolution of diseases in which oxidative stress has a critical role, so Tadalafil is currently being used in the treatment of pulmonary hypertension (Shen et al., 2020).

Radiation damage is caused by the overproduction of ROS, which devastates the levels of antioxidants, resulting in oxidative stress that damages cellular membranes, leading to lipid peroxidation (Mahgoub et al., 2020). A significant increase in lipid peroxidation as measured by MDA was observed 15 days after whole body irradiation in liver tissue, then an administration of Tadalafil completely reversed this oxidative stress induced by γ-rays in liver tissue, suggesting that it could protect its cellular membrane from radiation-induced lipid peroxidation and oxidative stress (Bektas et al., 2016).

The MPO activity is an indicator of quantitative inflammation and the infiltration of the hepatocytes with leukocytes (Pinar et al., 2018). In a recent study, the hepatic injury was assessed by an increase in MPO activity in hepatocytes indicating the liver inflammatory injury and hepatic oxidative stress in the irradiated group (Weiss et al., 2019). Tadalafil pre-treatment significantly ameliorated liver injury, made evident by a decrease in MPO activity. Tadalafil significantly reduced leukocyte infiltration and combat liver damage caused by ischemia and reperfusion injury in the rat model (Thakur et al., 2019).

Irradiation induced escalation of serum TNF-α and IL-6 levels that contribute to DNA damage and cell death (Wang et al., 2020). The current study demonstrated Tadalafil mediated enhancement of TNF-α and IL-6 levels in the irradiated animals group. However, this increase in the serum TNF-α and IL-6 level was significantly blocked by Tadalafil treatment when compared to the corresponding irradiated rats group. Suppression of serum TNF-α and IL-6 levels clearly indicates the anti-inflammatory effect of Tadalafil (França et al., 2019). Also, Wang et al. (2020) concluded that after irradiation, inflammatory reactions are developed in the organisms and they supposed that inflammation arises from activation of TNF-α, so its inhibition can be very useful in the treatment of radiation-induced diseases.

Gamma-rays-induced hepatic injury attained reduction in cellular antioxidant defence mechanisms; GSH content, and GPx, CAT and SOD enzymes activity (Abdel-Magied et al., 2019). In a recent work, liver tissue achieved decrease in GSH content, and GPx, CAT and SOD enzymes activity, which is indicative of cellular oxidative stress and liver dysfunction leading to enhanced production of ROS and reactive nitrogen species (Sayed et al., 2018). Tadalafil treatment significantly reversed all these antioxidant indices as a protective-treatment for liver-dysfunctions in the irradiated rats. In a recent study, it was shown that in rat nephropathy, Tadalafil acts by increasing the enzymatic and non-enzymatic antioxidant system capacity level of total GSH, GPx, CAT and SOD. This adaptive response could potentially control the production of ROS by modifying the oxidant/ antioxidant constancy induced by Tadalafil administration **(**Iordache et al., 2020).

Conclusion

Tadalafil may have a protective effect against gamma irradiation-induced liver dysfunction by antagonizing the free radicals generation and enhancement of the antioxidant defence mechanisms. It may have a promising approach for alleviating radiation-induced liver injury.

Recommendations: Further studies are necessary to clarify the mechanisms involved in the antioxidant response and the potential benefits of Tadalafil on the redox state for conserving the life of an animal.

Acknowledgements: The authors are thankful to their colleagues in Health Radiation Research Department for providing laboratory conveniences, and to the members of the National Center for Radiation Research and Technology (NCRRT), Egyptian Atomic Energy Authority for providing the necessary irradiation facilities. This work was performed at the NCRRT, Egypt.

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