Original Article

The Modulatory Role of Ashwagandha Root Extract on Gamma-Radiation-Induced Nephrotoxicity and Cardiotoxicity in Male Albino Rats

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ABSTRACT

This study had been initiated to investigate the modulatory role of Ashwagandha root extract against gamma radiation-induced nephrotoxicity and cardiotoxicity in male albino rats. Administration of Ashwagandha (100 mg/kg) for 7 days prior to whole body gamma-irradiation exposure (6Gy) minimized the hazardous effects of radiation by decreasing the levels of serum urea, creatinine and creatine phosphokinase (CPK) and lactate dehydrogenase (LDH) activities. Also, ashwagandha induced significant decreases in the levels of nitric oxide (NO) and malondialdehyde (MDA) and significant increase in the superoxide dismutase (SOD) activity in heart and kidney tissues compared to irradiated group. On the other hand, administration of ashwagandha prior to whole body gamma-irradiation induced non significant change in the levels of reduced glutathione (GSH). Based on our data, it is proposed that Ashwagandha extract might involve in the protection of renal and cardiac damage induced by gamma irradiation owing to its antioxidant capacity.

Keywords: Ashwagandha; gamma radiation; nephrotoxicity; cardiotoxicity; rats.

INTRODUCTION

Ashwagandha (Withania somnifera) is an herb that grows in India, Pakistan, Afghanistan, Spain, parts of the Middle East, Africa, and the Canary Islands. It is sometimes called "Indian ginseng." probably because it is employed as an adaptogen or tonic in Ayurvedic traditional medicine.[1] It is not related to "true" ginseng (P. ginseng, P. quinquifolium). The root is used medicinally, although the seeds, shoots, juice and leaves have all been used traditionally as well.[2]

Ashwagandha has been found to contain steroidal lactones called withanolides. Much of the pharmacological activity of Ashwagandha is attributed to the presence of these steroidal lactones.[1,3] In addition, the roots provide alkaloids, 18 fatty acids, beta-sitesterol, polyphenols and phytosterols. Ashwagandha approves physical and mental health, renews the body in debilitated conditions, and increase longevity.[4] Also, Ashwagandha is known to have anti-inflammatory,[5] antitumor,[6] antidiabetic,[7] antioxidant,[8] cardioprotective,[9] and antistress effect.[10]

In living tissues, the water molecule is a major target of ionizing radiation reactions in which OH°, H_2O_2, HO_2 and H° radicals are formed. Many different types of reactive oxygen species (ROS) are produced such as OH°, O_2 and H_2O_2. These species can damage bases and cause different adducts and degradation products. Ionizing radiation can cause strand breaks that are responsible for most of the lethal effects of such radiation.[11]

The aim of this work is evaluation of the possible protective role of Ashwagandha root extract as a natural product against gamma-radiation-induced nephrotoxicity and cardiotoxicity in male albino rats.

MATERIALS AND METHODS

Animals

Male Wistar rats (weighing120–150g) were obtained from the animal farm of the Egyptian Holding Company for Biological Products and Vaccines, Egypt. Upon arrival, the animals were allowed to acclimatize for 1 week before starting the experiment. Animals were kept under standard conditions and were allowed free access to a standard requirement diet and water ad. Libitum. Animals were kept under a controlled lighting condition (light: dark, 13h-11h). The animals' treatment protocol was approved by the animal care committee of the National Center for Radiation Research and Technology (NCRRT), Cairo, Egypt.

Irradiation

Whole-body gamma-irradiation was performed at the National Centre for Radiation Research and Technology (NCRRT), Atomic Energy Authority, Cairo, Egypt, using (137cesium) Gamma Cell-40 biological irradiator. Animals were irradiated at an acute single dose level of 6 Gy delivered at a dose rate of 0.012 Gy/s.

Experimental design

Male albino rats were divided into four groups, 10 rats in each. In the control group, rats were administered vehicle by tube for 7 consecutive days. The second group was administered Ashwagandha extract (100 mg /kg, by tube) for 7 consecutive days.[12] Animals in the third group were administered vehicle by tube for 7 consecutive days, then exposed to single dose γ-irradiation (6 Gy). The Fourth group was received an Ashwagandha extract (100 mg /kg, by tube) for 7 consecutive days, one hour later rats were exposed to single dose γ-irradiation (6 Gy). Twenty-four hours after the last specific treatment, animals were anesthetized with ether, and blood samples were obtained by heart puncture.

Biochemical assays

Twenty-four hours after the last dose of the specific treatment, animals were anesthetized with ether, and blood samples were obtained by heart puncture and serum was separated by centrifugation (Sorvall TC centrifuge, Hamburg, Germany) at 750g at room temperature for 10 min. Serum urea nitrogen, creatinine were determined according to the methods of Hallet and Cook[13] and Bonsenes and Taussky[14] , respectively. Serum creatine phosphokinase (CPK) and Lactate Dehydrogenase (LDH) were determined according to the methods of

Swanson and Wilkinson[15] and IFCC[16], respectively.

Hearts and kidneys were quickly excised, washed with saline, blotted with a piece of filter paper and homogenized in ice-cold 0.15MTris-KCl buffer (pH 7.4) to yield a 20% (w/v) homogenate using a Branson sonifier (250, VWR Scientific, Danbury, CT, USA). The homogenates were used for the determination of malondialdehyde (MDA) level, superoxide dismutase (SOD) activity, reduced glutathione (GSH) content, and nitric oxide (NO) level. The homogenates were centrifuged at 800 g for 5 min at 4ºC to separate the nuclear debris. The supernatant so obtained was centrifuged (Eppendorf AG, centrifuge 5804R, Hamburg, Germany) at 15000 g for 30 min at 4ºC to get the post mitochondrial supernatant which was used to assay superoxide dismutase (SOD) activity.

Reduced glutathione (GSH) and malondialdehyde (MDA) levels in heart and kidney homogenates were determined spectrophotometrically using the methods of Ellman[17] and Buege and Aust,[18] respectively. Nitric oxide (NO) was measured as the stable end product, nitrite, according to the method of Miranda et al.[19] The activities SOD was determined according to the methods of Minami and Yoshikawa.[20]

Statistical analysis

Results were expressed as mean ± SE. The intergroup variation was measured by t-test. Statistical significance was considered at $p < 0.05$.

RESULTS

The exposure of rats to gamma-irradiation induced significant increases in the levels of serum urea, creatinine, CPK and LDH activities compared to the control group. While serum levels of urea, creatinine, and CPK and LDH activities were decreased significantly when the animals treated with Ashwagandha prior to irradiation exposure compared to the irradiated group (Table 1).

Gamma-radiation induced significant increase in NO and MDA levels and significant decrease in SOD activity and GSH content in the kidney tissue compared to the control group. Administration of Ashwagandha before exposure to gamma-irradiation induced significant decrease in the levels of NO and MDA in the kidney tissues compared to the irradiated group. On the other hand, significant increase was observed in the level of reduced glutathione and SOD activity in kidney tissues compared to the irradiated group (Table 2).

In table (3) the data revealed that gamma-irradiation induced significant increase in NO and MDA levels and significant decrease in SOD activity in the cardiac tissues compared to the control group. On the other hand, the administration of Ashwagandha prior to gamma irradiation exposure significantly decreased the levels NO and MDA and significantly increased the SOD activity in heart tissues compared to the irradiated group. While no significant changes were observed in the level of glutathione in all treated groups in heart tissues.

DISCUSSION

It is well documented that whole body gamma-irradiation produces reactive oxygen (ROS) intermediates in mammalian tissues.[21-24] These reactive free radicals alter the metabolism of various organs and cause a series of biochemical and physical disturbances in the different biological tissues. Increased production of ROS leads to lipid peroxidation, oxidation of DNA and proteins as well as activation of pro-inflammatory factors.[25] ROS also affect the antioxidant defense mechanisms by reducing the intracellular concentration of GSH as well as SOD, Glutathione-S-transferase (GST), and catalase (CAT) activities.[26]

The data of our study recorded that whole body exposure to gamma irradiation (6Gy) elevated the level of MDA and enhanced NO formation and decreased the level of GSH and SOD activity in kidney and heart tissues. These results are in agreement with those recorded by Bai et al.[27,28] The increase in lipid peroxidation might be due to the increase in cell membrane permeability and leakage of enzymes from the cells into the intracellular space and into the blood.[28, 29]

Kataoka and Yamaoka[30] postulated that SOD changes superoxide anion into hydrogen peroxide (H_2O_2), catalase, and GPx detoxify H_2O_2 into H_2O and O_2 . Glutathione directly reacts with ROS, and GPx catalyzes the destruction of H_2O_2 and hydroxyl radical. This catalysis generate oxidized glutathione (GSSG) and finally reduced glutathione (GSH). The depletion of GSH has been shown to cause inhibition of glutathione peroxidase activity and resultant increase in lipid peroxidation.[31]

Similar results have been reported by Voevodskaya & Vanin [32] and Gorbunov et al.[33] which revealed that gamma-irradiation may enhance endogenous NO biosynthesis in liver, intestine, lung, kidney, brain, spleen or heart of the animals, presumably by facilitating the entry of Ca^{2+} ions into the membrane as well as the cytosol of NO-producing cells though irradiation-induced membrane lesions.[33] Nitric oxide is a volatile diatomic free radical that plays physiological roles in normal tissues. The enhancement of NO production following exposure to high dose (6Gy) of gamma rays was attributed to high levels of expression of the inducible nitric oxide synthase,[34] they suggested that DNA strand breaks caused by hydroxyl radicals formed inside the cells by gamma-irradiation, or strand breaks caused by radiation, plays an important role in the enhancement of NO production.

Our data agreed with previous studies, which reported that irradiation caused a significant increase in CPK and LDH activities[35] and significant increase in urea and creatinine.[36] The excessive production of free radicals and lipid peroxides might have caused the leakage of cytosolic enzymes such as lactate dehydrogenase, creatine kinase and phosphatases. Ramadan et al. [37] suggested that the increase in serum urea was due to the increase in glutamate dehydrogenase enzyme as a result of irradiation and this may increase carbamoyl phosphate synthetase activity leading to increase in urea concentration.

The results of the present study revealed that, the administraion of Ashwagandha prior to gamma-irradiation (6Gy) induced a significant increases in the activity of SOD and reduced glutathione (GSH) level and a significant dercrease in NO and MDA levels in cardiac and nephrotic tissues compared to the irradiated group. This is in accordance with previous studies10,[38-41] Previous study reported that Ashwagandha significantly reduced free radical oxidation in mice, while concurrently increasing the activity of antioxidant enzymes such as SOD, glutathione peroxidase in rats.[42] Also, Dhuley [43] reported that Ashwagandha reduced free radical activity in stress-induced animals. The lowest dose of Ashwagandha only stimulated the synthesis of antioxidants glutathione and catalase. The observed increase in glutathione levels might be due to its enhanced synthesis. It is speculated that the increase in glutathione reductase activity may account for maintaining glutathione in its reduced state.[44] Priyandoko et al.[45] suggested that the cells treated with Ashwagandha could be protected against toxicity by multiple mechanisms including reduction in the production of ROS, subsequent damage at DNA and mitochondrial level, and induction of cellular defense machinery.

Mohanty et al.[44] observed that vitamin E and Ashwagandha treatment

significantly restored lactate dehydrogenase and creatine phosphokinase activity compared to the isoprenaline control group, suggestive of their cardioprotective effect. Also, Ashwagandha restored the myocardial antioxidant status and maintained membrane integrity as evidenced by a decline in malonyldialdehyde levels[44]. The cardioprotective effect of Ashwagandha might be due to myocardial adaptive changes (augmentation of endogenous antioxidants) on chronic administration and restoration of the antioxidant status of the myocardium.

The present study revealed that Ashwagandha possesses no toxicity at a dose of 100 mg / kg and does not cause significant changes in biochemical parameters in serum and tissues of rats. These finding supported by the finding of Dhuley[46] and phale et al.[47] who showed that Ashwagandha is a safe herb and did not reveal any toxicity.

CONCLUSION

The results from the present investigation indicated that administration of Ashwagandha prior to whole body exposure to gamma-irradiation protects against radiation damage by inhibiting radiation induced GSH and SOD depletion, increasing MDA and NO levels in kidney and heart tissues. Based on our data, it is proposed that Ashwagandha extract may involve in the protection of renal and cardiac damage induced by gamma-radiation owing to its antioxidant capacity.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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Table 1. Effect of Ashwagandha on the levels of urea and creatinine, the activities of creatinine phosphokinase (CPK) and lactate dehydrogenase (LDH) in serum of male albino rats irradiated with 6 Gy of gamma-irradiation (IR).

parameter/ Groups	Urea mg/dl	Creatinine mg/dl	CPK IU/L	LDH IU/L
Control	0.354 ± 0.02	0.848 ± 0.06	1391 ± 166.8	1086.6 ± 132.2
Ashwagandha	0.47 ± 0.03*	0.928 ± 0.07	1679.4 ± 269.33	1203.8 ± 192.3
IR	2.04 ± 0.04*	5.042± 0.04*	3415.6±264.3***	2109.6±251.6***
Ashwagandha + IR	0.546 ± 0.03*	1.098 ± 0.09	1977 ± 462.97**	1289 ± 146.43**

Data are presented as mean ± SE, n= 6. *Significantly difference $P \leq 0.05$, **High significantly difference $P \leq 0.01$, ***Very high significantly difference $P \leq 0.001$

Table 2. Effect of Ashwagandha on the levels of nitric oxide (NO), Glutathion (GSH), Malondialdehyde (MDA), activity of Superoxide dismutase (SOD) in kidney tissues of male albino rats irradiated with 6 Gy of gamma-irradiation (IR).

Parameters	Control	Ashwagandha	IR	Ashwagandha + IR
NO µmol/g tissue	46.01 ± 2.24	50.96 ± 2.59*	51.72 ±0.66**	46.40 ± 2.53*
GSH µmol/g tissue	2.16±0.313	2.01 ± 0.127	1.75±0.131*	2.25 ± 0.40
MDA n mol/L	1.61 ± 0.45	1.49 ± 0.44	3.58 ±0.61**	2.88 ± 0.98**
SOD µg/g tissue	110.85±1.3	118.57 ± 1.20	51.04±0.7*	104.27±0.96**

Data are presented as mean ± SE, n= 6. * Significantly difference $P \leq 0.05$, ** High significantly difference $P \leq 0.01$, ***Very high significantly difference $P \leq 0.001$

Table 3. Effect of Ashwagandha on the levels of nitric oxide (NO), Glutathion (GSH), Malondialdehyde (MDA), activity of Superoxide dismutase (SOD) in kidney tissues of male albino rats irradiated with 6 Gy of gamma-irradiation (IR).

Parameters	Control	Ashwagandha	Irradiation	Ashwagandha + IR
NO µmol/g tissue	24.6 ± 1.08	28.9 ± 3.57	29.52±0.791**	25.1 ± 3.09*
GSH µmol/g tissue	1.732 ± 0.171	1.668 ± 0.113	1.812 ± 0.094	1.981 ± 0.3122
MDA nmol/L	2.04 ± 0.04*	5.042 ± 0.04*	15.6± 1.3***	4.6± 0.6**
SOD µg/g tissue	92.55 ± 2.1	93.78 ± 1.56	44.64 ± 1.4*	80.85 ± 2.52**

Data are presented as mean ± SE, n= 6. *Significantly difference $P \leq 0.05$, **High significantly difference $P \leq 0.01$, ***Very high significantly difference $P \leq 0.001$