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Salvia officinalis l. (sage) Ameliorates Radiation-Induced Oxidative Brain Damage In Rats

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ABSTRACT

The present study was designed to investigate the oxidative stress and the role of antioxidant system in the management of gamma irradiation induced whole brain damage in rats. Also, to elucidate the potential role of *Salvia officinalis* (sage) in alleviating such negative effects. Rats were subjected to gamma radiation (6 Gy). Sage extract was daily given to rats during 14 days before starting irradiation and continued after radiation exposure for another 14 days. The results revealed that the levels of thiobarbituric acid reactive substances (TBARS), protein carbonyl content (PCC) and nitric oxide (NO) content were significantly increased, while the activities of superoxide dismutase (SOD) and catalase (CAT) as well as the reduced glutathione (GSH) content were significantly decreased in the brain homogenate of irradiated rats. Additionally, brain acetylcholinesterase (AChE) as well as alkaline phosphatase (ALP), acid phosphatase (ACP) and lactate dehydrogenase (LDH) activities were significantly increased. On the other hand, the results showed that, administration of sage extract to rats was able to ameliorate the mentioned parameters and the values returned close to the normal ones. It could be concluded that sage extract, by its antioxidant constituents, could modulate radiation –induced oxidative stress and enzyme activities in the brain.

***Keywords*: gamma radiation/brain/ *Salvia officinalis* /Lipid peroxidation / antioxidants/ acetylcholinesterase**

INTRODUCTION

Damage of normal tissue is the most important limiting factor in radiotherapy. It is possible at least theoretically, to eradicate a localized tumor if it is subjected to a large dose of radiation, but, practically; there is always the danger of damaging normal tissues adjacent to the tumor. Recently; attempts have been made to modify this effect by the administration of therapeutic agents after irradiation but before the development of the damage [(1)]. Oxidative stress occurs due to excessive free radical production and/or low antioxidant defence, and results in chemical alterations of bio-molecules causing structural and functional modi?cations [(2)]. The generation of the reactive oxygen metabolites (ROMs) plays an important role in the pathogenesis of irradiation-induced tissue injury. There are an increasing evidences to suggest that many degenerative diseases, such as brain dysfunction, cancer, heart diseases, and weakened immune system, could be the result of cellular damage caused by free radicals, and antioxidants present in human diet may play an important role in disease prevention [(3,4)]. Plants have been the companion of man and formed the basis of useful drugs for the treatment of various ailments. The use of plants may be bene?cial in protecting against the radiation-induced damage, since they are less toxic than synthetic compounds at their optimum doses [(5)]. Therefore, screening of plants present a major avenue for the discovery of new radioprotective drugs.

Salvia officinalis, a plant endemic to the Mediterranean region is one of the most popular herbal remedy in the Middle East to treat common health complications such as cold and abdominal pain [6].The leaves of *Salvia of?cinalis* L. (sage) are well known for their antioxidative properties [7,8] and are used in the food processing industry, but are applicable also to the area of human health [9]. There are some reports that sage has been recommended through the centuries as restoratives of lost or declining mental functions [10,11]. Phytochemically, the whole plant contains several antioxidants that prevent peroxidative damage to hepatocytes such as water-soluble compounds; salvianolic acid A, salvianolic acid B and rosmarinic acid [12], tanshinone IIA [13] and several phenolic glycosides [14]. Accordingly, this study was designed to evaluate the role of sage extract, when given orally, in protecting brain of rats from oxidative stress and alteration of enzyme activities caused by exposure to ? -radiation (6 Gy).

MATERIAL AND METHODS

Plant material:

Dried leaves of sage were purchased from a local herb market.

Sage water extract (sage tea):

It was prepared by pouring 150 ml boiling water onto 2 g of dried grounded leaves and allowing it to steep for 5 min. [15].

Experimental animals:

This study was carried out on 24 adult male albino rats weighing 155 ±15 g, obtained from the Egyptian Organisation for Biological Products and Vaccines (VACSERA, Giza, Egypt). The rats were maintained under controlled humidity; temperature (25 ± 2°C) and light (12h light/ 12h dark). They were fed on standard commercial rodent pellet diet and free access to water.

Irradiation of animals:

Whole-body Gamma - irradiation was performed at the National Centre for Radiation Research and Technology (NCRRT), Cairo, Egypt, using a Gamma Cell-40 biological irradiator. Animals were irradiated at an acute single dose level of 6 Gy delivered at a dose rate of 0.46Gy/ min.

Experimental Protocol:

Rats were divided into four groups (n=6). Group I (Control group), included rats neither treated nor irradiated. Rats in group II (Irradiated group) were exposed to gamma radiation (6 Gy). In group III (Sage group), rats were given sage water extract instead of drinking water according to Lima et al. (2005) along the experimental period (28 days). Group IV (Sage Irradiated group) included rats that were given sage water extract instead of drinking water for 14 days before gamma irradiation, and the administration of the extract was extended after radiation exposure (14 days).

Estimation of biochemical parameters

All the biochemical parameters were measured in the brain homogenate on day 14 following gamma radiation exposure.

Brain tissue homogenate preparation:

Animals were sacrificed by decapitation and whole brains were removed and rinsed with ice-cold isotonic saline. Brain tissue samples were then homogenized with ice cold 0.1M phosphate buffer (pH 7.4) in a volume 10 times the weight of the tissue. The homogenate was centrifuged at 10,000×g for 15 min and aliquots of supernatant separated and used for biochemical estimations.

Biochemical analysis:

Determination of lipid peroxidation product namely thiobarbituric acid reactive substances (TBARS) was carried out according to the method of Yoshioka *et al.* [16]. Protein carbonyl content was measured spectrophotometrically according to the method of Smith *et al.* [17]. Nitric oxide (NO) was measured (nmole/g wet tissue) as stable end product, nitrite, according to the method of Miranda *et al.* [18]. Superoxide dismutase (SOD) and catalase (CAT) activities were determined following the methods of Minami and Yoshikawa [19] and Aebi [20], respectively. Reduced glutathione (GSH) content was determined spectrophotometrically according to the method of Beutler *et al.* [21]. Acetylcholinesterase (AChE) activity was measured according to the method of Ellman *et al.* [22]. Alkaline phosphatase (ALP), acid phosphatase (ACP) and lactate dehydrogenase(LDH) in brain tissue were determined by using commercial kits from (Biodiagnostic, 29 Tahreer St., Dokki, Giza, Egypt),based on the methods of Babson[23],Babson Ready [24] and Moss and Handerson [25] respectively. In all enzymatic determinants the proteins were evaluated according to Lowry *et al.* [26].

Statistical analysis:

The results are expressed as means ±SE. values were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. $P < 0.05$ was considered statistically significant.

RESULTS

As observed in table (1), the statistical analysis showed that the levels of brain TBARS ,PCC and NO were increased significantly in irradiated rats as compared to those of control rats ($P < 0.05$). Pre- and post-treatment of rats with *Salvia officinalis* extract provided a marked normalization of brain tissue TBARS, PCC and NO levels as compared with those of irradiated rats ($P < 0.05$). Sage tea treatment of normal rats, however, did not modify the basal TBARS, PCC and NO levels which remained similar to those of control groups ($P < 0.05$).

Table 1: Brain thiobarbituric acid reactive substances (TBARS), protein carbonyl content (PCC) and nitric oxide (NO) concentration in the different animal groups.

Animals Groups	TBARS (n mol/ g protein)	PCC (n mol/mg protein)	NO (n mol / g protein)
Control	397.80±7.85^{c}	4.00±0.71^{c}	23.12±1.25^{c}
Radiation	478±10±8.99^{a}	5.83±0.56^{a}	45.00±3.14^{a}
Sage tea	388.54±5.00^{c}	3.98±1.03^{c}	23.60±1.27^{c}
Sage tea+ Radiation	413.04±7.87^{b}	4.30±0.66^{b}	34.65±1.98^{b}

Values are expressed as means of 6 records ± Standard Error

Means with different superscripts are significantly different at the 0.05 level

Regarding antioxidants, the data indicated that, ?-irradiation of rats induced a significant reduction in brain SOD and CAT activities as well as GSH content compared to normal control group (table 2). Administration of sage tea for 14 consecutive days prior to- and 14 days post-irradiation of rats resulted in a signi?cant increase in the activity of brain SOD and CAT and the content of GSH compared with those of irradiated animals ($P < 0.05$). However, the similar treatment of rats with *Salvia officinalis* extract in normal rats did not modify the basal levels of GSH content and SOD and CAT activities compared with control group ($P < 0.05$).

Table 2: Brain superoxide dismutase (SOD) and catalase (CAT) activities and reduced glutathione (GSH) level in the different animal groups.

Animals Groups	SOD (U /g tissue)	CAT (U/g tissue)	GSH (mg /g tissue)
Control	7.51±0.17 [a]	5.74±0.28 [a]	26.37±0.95 [a]
Radiation	5.38±0.25 [c]	3.60±0.24 [c]	13.63±0.94 [c]
Sage tea	7.57±0.21 [a]	5.51±0.24 [a]	28.41±1.97 [a]
Sage tea+ Radiation	6.63±0.16 [b]	4.52±0.19 [b]	20.37±1.45 [b]

Legend as table 1

As shown in table (3), in irradiated rats, there were significant elevations in brain ALP, ACP, LDH and AChE activities compared to normal control ones. The administration of sage extract before and after irradiation of rats signi?cantly limited the elevation in brain ALP, ACP, LDH and AChE activities.

Table 3: Brain alkaline phosphatase (ALP), acid phosphatase(ACP), lactic dehydrogenase (LDH) and acetylcholinesterase(AchE) activities in the different animal groups.

Animals Groups	ALP (U/100 mg tissue)	ACP (U/100 mg tissue)	LDH (U /mg protein)	AchE (n mol /mg protein)
Control	65.28±4.75 [c]	85.20±1.02 [c]	5.74±0.20 [c]	138.50±7.12 [c]
Radiation	93.19±8.11 [a]	126.13±2.78 [a]	8.52±0.52 [a]	363.97±11.00 [a]
Sage tea	63.38±5.29 [c]	83.79±5.41 [c]	5.70±0.19 [c]	142.26±2.01 [c]
Sage tea+ Radiation	74.20±3.71 [b]	93.70±2.35 [b]	7.41±0.37 [b]	183.60±9.88 [b]

Legend as table 1

DISCUSSION

During radiotherapy normal brain can undergo undesirable tissue injury especially in the treatment of cerebral tumors. The use of complementary medicines, such as plant extracts, in dementia therapy varies according to the different cultural traditions. Traditional medicine systems have long included a number of Lamiaceous and Asteraceous plants for use in treatment of a variety of disorders. Members of the sage family, such as *Salvia officinalis* and *Salvia lavandulaefolia*, have a long history of use as memory-enhancing agents coupled with cholinergic properties that may be relevant to amelioration of the cognitive deficits associated with Alzheimer's disease [(11)].

In the present study, irradiation of rats significantly induced lipid peroxidation and protein oxidation and increased NO levels and reduced antioxidant defense indicating increased oxidative-nitritive stress. The increased levels of TBARS (an index of lipid peroxidation) in brain tissues of ?-irradiated rats, may be due to the free radical attack on cell membrane phospholipids and circulating lipids and, thus, TBARS acts as a sensitive biomarker for oxidative stress that occurs as part of the pathogenesis of various diseases [(27,28)]. These results are in agreement with previous studies,which have shown that free radicals caused lipid peroxidation in the irradiated tissue [(29,30)]. Moreover, Manda *et al.* [(31)] examined a wide variety of tissues such as brain, liver, spleen, kidney and testis after exposing the rats to the whole body radiation of 4 and 6 Gy and they found that among all the tissues, brain is the most susceptible to radiation-induced changes in lipid peroxidation.

Carbonyl groups, a biomarker of protein oxidation, are produced on protein side chains when they are oxidized [32]. The damage to cellular proteins by oxygen free radicals formed during normal aerobic metabolism or as a result of action of exogenous factors (radiation, oxidants, etc.) plays an important role in cell aging and death and underlies pathogenesis of many diseases [33]. The observed increase in brain carbonyl contents (Table 1) is an indicator for cellular protein damage by gamma-irradiation. In addition, the enhanced nitric oxide production (Table 1) in the brain of irradiated rats could be due to direct DNA damage where DNA damage activated poly (ADP-ribose) polymerase (PARP), which induced nuclear factor kappa B (NFkB) activation, ?nally resulting in increased iNOS expression and NO production [34] (Table 1).

Some studies have reported that NO is an important mediator of radiation- induced acute tissue damage [35]. Nitric oxide is a transient gaseous second messenger molecule functioning in vascular regulation, immunity, and neurotransmission. NO levels are associated directly with the development of brain injury in strokes and other neuropathological disorders in humans [36].The present results show that whole body gamma-irradiation of rats at 6 Gy enhanced the formation of NO(x). Similar results have been reported by Gorbunov et al. [37]. 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 [38]. To control the flux of ROS, aerobic cells have developed their own defense system, the antioxidant system, which includes enzymatic and non-enzymatic components. The antioxidant system consists of low molecular-weight antioxidant molecules, such as glutathione (GSH) and various antioxidant enzymes. Superoxide dismutase (SOD), the first line of defense against oxygen-derived free radicals, catalysis the dismutation of superoxide anion into H_2O_2. H_2O_2 can be transformed into H_2O and O_2 by catalase (CAT). Glutathione peroxidase (GSHPx) reduces lipidic or nonlipidic hydroperoxides as well as H_2O_2 while oxidizing GSH [39]. The decrease in the activities of SOD and CAT and the decreased level of GSH in the brain tissues may be due to their utilization by the enhanced production of ROS [40]. A significant decrease in GSH content in whole brain was observed following gamma irradiation (5Gy) by Ahaskar and Sisodia [41]. In agreement with these ?ndings, Sandeep and Nair [42] observed a signi?cant depletion in the antioxidant system, and an increased lipid peroxides in animals exposed to whole body ?-irradiation. The decreased levels of GSH in brain of irradiated animals may have resulted from the activity of GST and glutathione peroxidase (GPx) in reducing lipid hydroperoxide to stable non-radical lipid alcohols or GSH is directly utilized as an antioxidant in terminating free radical reaction initiated by irradiation [43].

The data obtained by the present study illustrated (Tables1,2) further indicated that administration of water extract of *S. officinalis* (sage tea) to irradiated rats caused a significant decrease in the level of TBARS, protein carbonyl and NO in brain tissue and elevated the SOD and CAT enzymes activities and GSH contents when compared with irradiated rats. Generally, the antioxidant effects of sage extracts have often been attributed to phenolic and monoterpenic compounds [44]. Flavonoids are a diverse group of polyphenols [45] rosmarinic acid being the most representative that possess several modulatory effects, either inducing or decreasing the expression of SOD and CAT enzymes depending on structure, concentration, and assay conditions. Rosmarinic acid is the predominant phenolic compound in sage [18] and its effects was attributed to the compound's antioxidant properties acting as scavenger of reactive oxygen species [46]. Additionally, the protection of cell viability conferred by sage extracts seemed to be due, mainly, to their ability to prevent GSH depletion by their main phenolic compounds, rosmarinic acid and luteolin-7-glucoside. Nevertheless, unknown compounds other than phenolics also seem to contribute to the antioxidant effects of sage on basal GSH levels [47]. However, Lima et al., [47] and Brandstetter et al. [48] showed the ability of sage (mainly the methanolic extract) to increase basal GSH levels, probably by the induction of glutathione synthesis.

Cholinesterases are a large family of enzymatic proteins widely distributed throughout both neuronal and non-neuronal tissues. In the present study, irradiation treatment of rats was found to significantly elevate acetylcholinesterase activity, an enzyme responsible for degradation of acetylcholine (Table 3). Schwenke et al., [49] showed that ?- irradiation of erythroleukemic K562 cells caused an increase in acetylcholinesterase activity accompanied by cell differentiation and cessation of cell proliferation. This increase in acetylcholinesterase activity was, however, significantly restored by *Salvia officinalis* extract administration to irradiated rats. However, it has been reported that *Salvia officinalis* has CNS cholinergic receptor binding activities that may be relevant to enhance or restore mental functions including memory [50], at least for AChE, suggesting that relevant components of Salvia can cross the blood– brain barrier and increase cholinergic transmission via cholinesterase inhibition [51]. Recently, Sidharth et al., [52] suggested that sage administration can modulate cholinergic neurotransmission and/or prevent cholinergic neuronal loss.

The present study illustrated that ?- irradiation of rats led to a significant elevation in brain lactate dehydrogenase (LDH), alkaline phosphatase (ALP) and acid phosphatase (ACP) activities (Table 3). LDH serves as a very important metabolic enzyme in brain cells and is released into the blood stream from injured brain cells. Thus, LDH level in serum or brain cell is a reliable index to evaluate cerebral ischemic injury [53]. Alkaline phosphatase is a membrane-associated enzyme, which predominantly concentrated in the vascular endothelium in the brain. There is a more or less continuous sheath of ALP covering all internal and external surfaces of the central nervous system including the spinal cord and thus it may functionally be part in the blood-brain barrier mechanism. On the other hand, intracellular ACP is largely confined to lysosomes, which primarily respond to cellular injury. The increased activity of LDH activity in brain homogenate of irradiated animals is in accordance with the findings of Abd-El-Fattah et al. [54]. In the present study, the increase in acid phosphatase level is similar to the observation of Singh et al. [55] and may be attributed to the rupture of lysosomal membrane in the gamma irradiated rat brain. In the present work, administration of sage tea to rats caused a pronounced reduction in the elevated activities of LDH, ALP and ACP in irradiated rats. Such decrease could be due to the antioxidant properties of sage constituents as polyphenols (carnosol, carnosic acid, and rosmarinic acid) and flavonoids (apigenin) that protect cellular membranes integrity from radiation induced oxidative damage and repair the antioxidant system [56], consequently, improve brain structure and function against radiation exposure.

CONCLUSION

In conclusion, the current study revealed that sage extract possibly has a protective role against ?-radiation induced oxidative stress and alteration of enzymes activities in the brain. Therefore, the receipt of *sage* may encourage patients undergoing radiotherapy to improve general health condition.

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