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Original Article

DIETARY SUPPLEMENTATION OF NATURAL AND SYNTHETIC PRODUCTS REDUCES ANXIETY IN MICE AGAINST ELECTRON BEAM RADIATION INDUCED OXIDATIVE STRESS

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Abstract :

Background : Due to the increased use of ionizing radiation in various aspects of human life, free radical formation is greatly augmented during exposure. This causes damage to the central nervous system. The natural and synthetic products have proven therapeutic benefits. Nardostachys jatamansi, an indigenous medicinal plant showed to promote physical and mental health augment resistance of the body against disease and has shown potent antioxidant activity. 1, 2, 4 triazole derivatives are the synthetic organic compound which has various pharmacological activities.

Objectives : To study the anxiolytic and protective effect of 100mg of ethanolic extract of Nardostachys jatamansi (NJE) and triazole (AMT) on the mice exposed to 6Gy Electron beam radiation (EBR).

Materials and Methods : The animals were treated with 100mg of NJE and Triazole for 15 days before radiation exposure. The anxiety status of animals observed once for every 3 days during experiment period. The biochemical estimations were carried 15 days after irradiation in mice brain homogenate.

Results and Conclusion : Treatment of mice with phytochemical and synthetic compound before irradiation caused a significant depletion in anxiety, lipid peroxidation followed by significant elevation in catalase, total antioxidant, and total protein. Our results indicate that the protective activity of NJE and triazole on radiation induced anxiety and oxidative stress may be due to free radical scavenging and increased antioxidant level in mice.

Keywords : Free radicals, Anxiety, Electron beam, Oxidative stress.

Introduction :

Exposure to ionizing radiation increases production of Reactive oxygen species and can lead irradiated cells into the state of oxidative stress, which has been implicated in an enormous variety of natural and pathological processes [1]. Oxidative stress on nervous tissue can produce damage by several interacting mechanisms. This may alter variety of central nervous system (CNS) - mediated processes [2].

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Anxiety disorders are the highly prevalent psychiatric disorders, affecting an estimated 25% of the adult population at some point during their lifetime [3]. It has been demonstrated that flavonoids possess mild sedative and anxiolytic effects. The naturally occuring flavonoids and their synthetic derivatives have been reported to selectively bind to the central benzodiazepine receptors, and to exert anxiolytic and other benzodiazepine-like effects in animals [4].

Nardostachys jatamansi (family Valerianaceae), an indigenous medicinal plant induces in organism a state of resistance against stress. It helps to promote physical and mental health augment resistance of the body against disease and has shown potent antioxidant activity. It has also shown marked tranquillizing activity, as well as hypotensive, hypolipidemic, anti-ischemic, antiarrhythmic, hepatoprotective, anticonvulsant,

neuroprotective activities [5-7].

Triazoles are the important class of heterocyclic compounds having three nitrogen atoms. They are of two types, 1, 2, 3 triazoles and 1, 2, 4 triazoles. Various 1, 2, 4 triazoles and its derivatives are found to be linked with diverse pharmacological activities. Compounds containing 1,2,4-triazole ring have been reported to possess different biological activities such as antimicrobial [8], antifunga l [9], anti-inflammatory [10], antiviral [11], anticancer [12, 13], analgesic [14], and anticonvulsant [15] activity depending on the substituent in the ring system.

In the present study, the potential and modulatory role of Nardostachys jatamansi and Triazole derivative has been explored on radiation induced changes in anxiety and oxidative stress in mice.

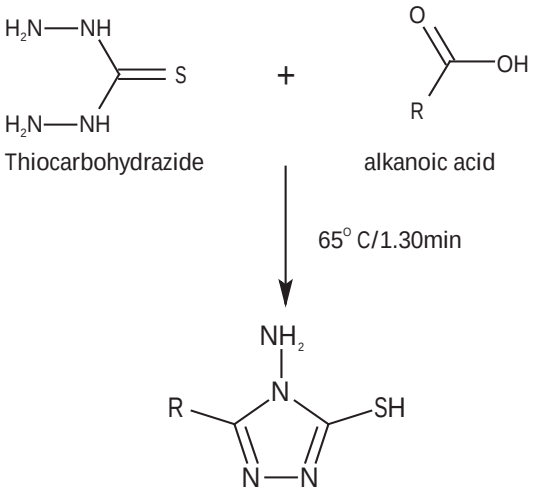
Materials and Methods :

Plant material and preparation of extract:

The plant material i.e. rhizome powder of Nardostachys jatamansi was collected from GENUINE chemical co, Mumbai. This powder was extracted with 95% ethanol at room temperature, concentrated in reduced temperature and pressure on rotary evaporator and stored at 4°C.

Chemistry

4-amino-5-mercapto 1, 2, 4-triazoles was prepared according to the methods proposed in literature [16].



Thiocarbohydrazide + alkanoic acid

4-amino-5-mercapto-3-substituted-1, 2, 4-triazole

Where,R=H for AMT

Animal care and handling:

Animal care and handling was carried out according to the guidelines set by WHO (World Health Organization; Geneva, Switzerland). The institutional animal ethical committee has approved this study. Swiss albino mice aged 6 -8 weeks and weighing 25±5 g, taken from an inbred colony, was used for this study. The mice were maintained under controlled conditions of temperature and light (light: 10 h; dark: 14 h). The animals were housed in a polypropylene cage containing sterile paddy husk (procured locally) as bedding throughout the experiment. They were provided standard mouse feed and water ad libitum.

Irradiation :

The irradiation work was carried out at Microtron centre, Mangalore University, Mangalore, Karnataka, India. The animals were restrained in well-ventilated perspex boxes and exposed to whole-body electron beam at a distance of 30 cm from the beam exit point of the Microtron accelerator at a dose rate of 72 Gy/min.

Experimental protocol:

The following groups of animals were used.

Group I: Control

Group II: Animals were exposed to 6Gy (sub-lethal dose) electron beam radiation (EBR).

Group III: Animals were received on N.jatamansi extract (100mg/kg body weight) orally for 15 days (The required amount of NJE was dissolved in 10%DMSO). 1 hour after final dose animals were exposed to 6Gy EBR.

Group IV: Animals were received on triazole (AMT) (100mg/kg body weight) orally for 15 days (The required amount of AMT was dissolved in dist water). 1 hour after final dose animals were exposed to 6Gy EBR.

Elevated Plus Maze Model [17]

The plus-maze apparatus, consisting of two open arms (16 x 5 cm) and two closed arms (16 x 5 x 12 cm) having an open roof. Both the drugs (100 mg/kg) were administered orally for 15 days once daily and the last dose was given on the 15th day, 60 min prior to radiation exposure. The mouse

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was placed at the center of the maze with its head facing the open arm. During the 5 min experiment, the behavior of the mouse was recorded as: the number of entries into the open or closed arms and time spent by the mouse in each of the arms. An arm entry was defined as the entry of all four paws into the arm. The animals were observed once for every 3 days during experiment period.

Dissection and homogenization of Brain

On 15th day post irradiation, animals were scarified by cervical dislocation, followed by decapitation. The whole brains were removed and 10% (W/V) tissue homogenates were prepared in 0.4M phosphate buffer pH7.0, centrifuged for 25 min at 5000 rpm at 4°C and the supernatant was used for estimation of the following biochemical assays.

Lipid peroxidation (LPx):

LPx was measured by the method of Beuege and Aust [18]. Briefly, serum was mixed with TCA-TBA-HCl and was heated for 15 min in a boiling water bath. After centrifugation the absorbance was recorded at 535 nm using a UV-Vis double beam spectrophotometer. The LPx has been expressed as Melondialdehyde in µM per liter.

Total antioxidant capacity:

Total antioxidant capacity of serum was determined by the phosphomolybdenum method as described by Prieto et al. [19]. The serum was precipitated with 5% TCA, it was then made to react with TAC reagent containing phosphomolybdenum at 95°c for 90 min. The absorbance was read at 695nm.

Catalase activity:

Catalase activity in RBC was measured spectrophotometrically as previously described [20]. The method is based on the fact that catalase causes breakdown of H_2O_2 (30mM). The H_2O_2 was mixed in 3ml of Phosphate buffer (pH 7.0) and then 50µl of 1:20 diluted erythrocyte was added and the changes in absorbance at 240 nm were recorded up to 2 min at the interval of 15sec. The enzyme activity was expressed as Units mg Hb^{-1}.

Total protein

The total protein in the brain tissue was estimated by biuret method [21]. 20µl of tissue homogenate was mixed with 1ml of reagent. 5 min after incubation the absorbance was read at 546nm. The results were expressed as gram protein per 100ml of 10%homoginate.

Statistical analysis

Results were expressed as Mean ± standard deviation. Comparison between the control and treated groups were performed by analysis of variance (ANOVA), followed by student's t-test. In all the test, criterion for statistical significance was $P<0.05$.

Results :

The test conducted in the elevated plus maze had shown positive results. The irradiated animals showed spending more time in closed arm than the open arm. The time spending in closed arm can be directly considered as the level of anxiety. We also observed, there was an increase in level of anxiety from the day of irradiation to the 15th day after irradiation. The treatment groups, both NJE and Triazole have showed decreased level of anxiety when compared to irradiated group (Table 1).

The irradiation of mice to 6Gy of electron beam radiation induces lipid peroxidation. The irradiated group had showed significant increased Melondialdehyde (MDA) level and decrease in the catalase, total protein and antioxidant level. But the pre supplementation of NJE and AMT before irradiation had showed the decreased level of lipid peroxidation and increased level of catalase, total protein (Graph 1), antioxidant when compared to irradiated group (Table 2). This proves that the synthetic and natural product with antioxidant property helps in lowering the oxidative stress in irradiated mice.

Discussion :

Anxiety may be regarded as a particular form of behavioral inhibition that occurs in response to environmental events that are novel. It has been established that there are lot of plant secondary metabolites being employed in the treatment of psychotic disorders especially for anxiety in

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Table 1 : Effect of NJE and AMT on animals in EPM model

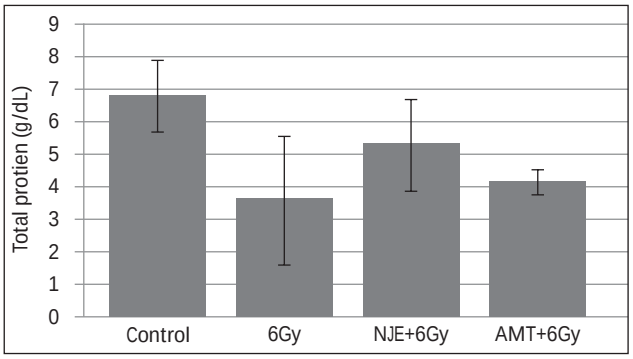
	No.of entries to open arm		No.of entries to closed arm	
	Before Irradiation	15th day after irradiation	Before Irradiation	15th day after irradiation
6Gy Irradiated animal	12.50±2.10	2.50±1.09	9.90±1.10	5.53±1.50
NJE treatment	10.75±2.15	6.80±2.55	11.12±2.10	5.00±1.75
AMT	12.10±2.70	7.10±1.25	10.25±1.55	6.23±1.65

Values are expressed as Mean ± Standard deviation. P<0.05 as compared to treated group with 6Gy irradiated radiation group.

	Control	6Gy irradiation	NJE treatment +6Gy irradiation	AMT treatment +6Gy Irradiation
MDA (µM/g tissue)	3.74±0.51	14.77±7.89*	10.30±3.20**	10.40±4.25**
Catalase (U/mg protein)	8.35±2.10	2.11±1.09*	7.23±2.15**	5.45±1.11**
TAC (µg/ml 10% homoginate)	88.80±31.30	39.80±11.12*	82.25±10.33**	69.45±9.35**

Values are expressed as Mean ± Standard deviation. **P<0.01 as compared to the 6Gy irradiated radiation group. *P<0.05 as compared to 6Gy irradiated radiation group with control.

Graph 1 : Effect of Treatment with NJE and AMT on Total Protein Levels in Brain of Mice Exposed To Electron Beam Radiation



Values are expressed as Mean ± Standard deviation. P<0.05 as compared to treated group with 6Gy irradiated radiation group.

traditional medicine practice. The results of present study show that extract of Nardostachys jatamansi exhibited anxiolytic activity. Recently, several herbal constituents have been reported to possess anxiolytic effects through animal models of anxiety [22], such as *Spondias mombin* [23], *Trigonella foenum-graecum* [24], *Sapindus mukorossi* [25], *Panax ginseng* [26]. Other than the natural products synthetic compounds such as Nitrazepam [27], Baicalin [28] have also showed anxiolytic property.

A single whole-body exposure of mammals to ionizing radiation results in a complex set of syndromes whose onset, nature and severity are a function of both total radiation dose and radiation quality. At the cellular level, ionizing radiation can induce damage in biologically important macromolecules such as DNA, proteins, lipids and carbohydrates in various organs [29,30].

Nardostachys jatamansi root extract has shown both *in vitro* and *in vivo* antioxidant property [31]. It attenuates stress induced elevation of biochemical changes such as membrane lipid peroxidation, elevated NO production in brain as well as stomach, levels of antioxidant enzymes like catalase, which are consistent with its anti stress properties. The similar mechanism might be the reason for the protection of mice against electron beam radiation induced lipid peroxidation followed by oxidative stress.

Depletion of intracellular catalase, total protein and total antioxidant level has been implicated as one of the causes of radiation induced damage, while increased levels of this are responsible for the radioprotective action. Pre supplementation of NJE and AMT helped to restore the TP, TA and catalase level when compared to the concurrent irradiation control group. This inhibits the radiation induced lipid peroxidation, thereby protecting against radiation-induced damage.

Conclusion :

From the above observations the study conclude that ethanolic extract of *Nardostachys jatamansi* and synthetic triazole compound possesses anxiolytic activity, also it showed protective effect against radiation induced oxidative stress. However further studies are required to know the exact mechanism of action.

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