RADOPROTECTIVE EFFECT OF AN EGYPTIAN WILD HERB AMBROSIA MARITIMA L. (DAMSISSA): BIOCHEMICAL STUDY ON NEUROTRANSMITTERS

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Ionizing radiations as gamma irradiation (GI) are frequently used in the treatment of different tumors as endocrine malignancies. These radiations were accompanied by severe side effects and elevated mortalities. Of the most common deleterious consequences, are mood disturbances which are reflections of neurotransmitter disturbances. In the present study, we studied the effect of GI and tested an alcoholic extract of Ambrosia maritima L. (Damsissa), (DE) on nor adrenaline (NA), dopamine (DA), serotonin (5-HT), histamine (HA), calcium (Ca), sodium (Na) and potassium (K) serum levels in three groups of Wistar albino rats, classified into : DE-treated, whole-body GI and DE-pre-irradiated groups, compared with a control group (n = 10). The DE-treated groups were given an alcoholic extract of leaves equivalent to 15 g leaves/kg body weight per orally (PO) as a single daily dose for 5 consecutive days. Whole-body GI was a single 5 Gy dose. All blood samples were withdrawn from fasting animals 24 hours after dosing. The results revealed a significant decrease in serum NA, DA, 5-HT and HA with increased Ca and Na levels in GI subjects. Pre-treatment with DE significantly elevated NA, decreased Ca but increased Na and K levels. Treatment with DE alone, significantly increased both NA and DA, but decreased Ca with increased Na and K levels. These results demonstrated that GI most probably decreases NA, DA, 5-HT and HA serum levels through decreasing Ca influx into cells, which probably inhibited K-channel activity. This action, presumably lowered neurotransmitter exocytosis.

DE pre-treatment decreased Ca and increased both Na and K levels with a consequent elevation of NA and DA levels in relation to GI-effect. We could conclude that GI elicits its mood disturbances probably through disturbing NA, DA, 5-HT and HA release as an outcome of K-channel disturbance, with non significant impact on Na levels. DE is recommended as an economic radioprotective pre-medication.

Ionizирующие излучения, такие как γ-радиация, часто используются для лечения различных опухолей, в том числе злокачественных эндокринных. Такие облучения сопровождаются тяжёлыми побочными эффектами и повышенной смертностью. Среди наиболее общих последствий следует отметить нарушения настроения, отражающиеся нарушения нейротрансмиттеров. В настоящей работе изучены эффекты γ-облучения и спиртового экстракта Ambrosia maritima L. (Амброзия) на уровне норадреналина, допамина, серотонина, гистамина, а также кальция, калия и натрия в сыворотке в трех группах крыс-альбинос Wistar. Крысы были поделены на контрольную и три исследуемые группы по 10 штук: принимавшие экстракт, облученные по всему телу, принимавшие экстракт и затем облученные. Спиртовый экстракт листьев Ambrosia maritima L. испытуемым группам давали однократно ежедневно парентерально в течение пяти дней из расчета 15 г листьев на килограмм массы тела за прием. Мышей облучали разовой дозой 5 Грея. Все образцы крови были отобраны у голодных мышей через 24 часа после облучения. Результаты демонстрируют значительное снижение уровня норадреналина, допамина, серотонина и гистамина в сыворотке, а также возрастание концентраций кальция и натрия в облученных объектах. Предварительное введение экстракта Ambrosia maritima L. значительно повышает уровень норадреналина, снижает концентрацию кальция, но повышает концентрации калия и натрия. Под действием только экстракта без облучения значительно возрастают уровни норадреналина и допамина, концентрация кальция снижается, а натрия и калия – возрастает. Полученные результаты показывают, что γ-облучение, наиболее вероятно, снижает уровень норадреналина, допамина, серотонина и гистамина в сыворотке вследствие снижения притока кальция в клетки, а последний, вероятно, ингибирует активность калиевых каналов. Этот эффект, вероятно, снижает нейротрансмиттерный экзоцитоз.

Предварительное введение экстракта Ambrosia maritima L. уровня кальция снижает, а калия и натрия – увеличивает с последующим возрастанием уровней норадреналина и допамина по сравнению с эффектом γ-облучения. Предполагается, что нарушения настроения после γ-облучения, вероятно, связаны с нарушениями высвобождения норадреналина, допамина, серотонина и гистамина вследствие нарушения калиевых каналов с незначительным влиянием уровня натрия. Спиртовый экстракт листьев Ambrosia maritima L. рекомендован в качестве экономичного радиопротекторного пре-медикатора.

* Автор, с которым следует вести переписку.
Introduction

Gamma irradiation (GI) is one of the ionizing radiations used for the treatment of tumors, specially for endocrine glands. The basic mechanism for cell – death induced by irradiation is programmed cell apoptosis. This arrest to cell cycle was always accompanied by deleterious side effects [1]. The study of neurochemical effects of exposure to GI seems to be an important issue that may contribute to better understanding of the underlying causes of mood disturbances (MD) following radio therapeutic sessions. The early neuro-vegetative syndrome observed in irradiated subjects was attributed to activation of dopamine (DA) – dopamine receptor binding [2]. In addition, plasma histamine (HA) level was elevated by whole – body irradiation with subsequent disturbances in cerebral blood flow and systemic blood pressure [3]. Moreover, ionizing radiation was reported to alter the response of noradren-ergic terminals which was attributed to an increase in cerebellar nor adrenaline (NA) content [4]. Also, GI modified the effects of acetylcholine and calcium (Ca) on potassium (K) permeability [5]. However, other radiation tools as ultraviolet irradiation although induced a tendency to Ca accumulation, it didn't alter both sodium (Na) and K storage in the whole rat body [6].

Although radiotherapy is a common therapeutic line for the treatment of different tumors, frequent mortalities were reported for patients and even radiologic technologists [7]. Western herbalism relied on ancient civilizations of Egyptians, Greeks and Romans. It also utilized the Islamic, Indian and Tibetan cultures in herbal remedy [8]. The need for economic, less toxic and more common phytoprotective alternatives against GI-induced sequela directed many researchers to test different locally available herbs as radioprotectives. Many of the tested plants proved efficient and valuable role in radioprotection as Podophyllum [9], Hippophate [10], Tinospoa [11], and Ginseng [12]. Ambrosia maritima L. (Damsissa) is one of the wild plants present in Egypt and different African countries of the Nile valley. It belongs to the subfamily tubuliflora which is a branch of the family Compositae of flowering plants. It contains important sesquiterpene lactones and flavonoids which showed molluscicidal and cytotoxic activities [13]. The most active ingredients of this plant are ambrosin and damsin [14]. Ambrosin belongs to a group of natural products known as pseudoguaianolides, it was totally synthesized and described by Grieco et al at 1982 [15]. Damsin is 2,3-dihydroambrosin, however, ambrosin has the following structural formula:

![Chemical Structure of Ambrosin](image)

Drinking decoctions of Damsissa were the most commonly used remedy for schistosomiasis in Upper Egypt [16]. Aqueous-methanolic extract of the plant showed antioxidant and hepatoprotective actions in drug-induced hepatotoxicity in rats [17].

The radioprotective effect of this plant wasn't tried before; so, in the present study we focused my interest on the possible radioprotective utility of this wild plant when given to GI rats. This may contribute to: (1) a better understanding of the biochemical pathways underlying the post-radiation neurologic symptoms through blood (not tissue) investigations and (2) looking for an economic radioprotective medication that may add to its hepatoprotective and antibilharzial actions. This may be valuable for river valleys-populations like most Africans.

Materials and methods

Animals. Forty male Wistar rats were maintained on a 12 hours light / dark cycle, given free access for mineral water and controlled diet of barley and carrots. The weight of the animals were ranging from 110–120 g, they were classified into four groups, each group consisted of 10 rats.

Treatment with irradiation and the plant extract. The first group served as control (given only the solvent,50 % ethanol, orally by stomach tube).The second group was given a single daily dose of an alcoholic
extract of *Ambrosia maritima*, extracted by 50% ethanol, orally for 5 consecutive days (equivalent to 15 g leaves / 1 kg body weight/day), (Damsissa extract = DE), [18]. The third group was exposed to whole body GI as a single dose of 5 grays (Gy), taking into account that the Gy is the radiation unit equivalent to an energy of one joule / one kilogram of target mass [19]. The fourth group was given 5 doses of DE (one dose / day) for 5 consecutive days, then exposed to a single whole body GI (5 Gy).

**Sampling:** Morning fasting blood samples were withdrawn 24 hours after irradiation, centrifuged and sera were kept at –80 °C for analytical procedures.

**Methods:** Both (NA), (DA) and serotonin (5-HT) were fluorometrically determined using serum samples instead of tissue reported in the original method [20]. Histamine (HA) was determined also fluorometrically according to the method of Vidal – Carou et al [21]. Calcium was spectrophotometrically determined [22]. Both Na and K were determined by flame photometry [23].

**Statistical analysis:** The obtained data were analyzed by one – way ANOVA utilizing computerized statistical program. P values < 0.05 were considered significant.

**Results**

**Biogenic amine variations (table 1):** NA level was significantly increased by administration of DE (p < 0,01). GI significantly decreased NA level (p < 0,001), pretreatment of irradiated rats by DE restored NA level nearly to normal. DA level was significantly increased (p < 0,01) by DE. GI significantly decreased DA level (p < 0,01), but pretreatment with DE only decreased DA level less significantly (p < 0,05). 5-HT was significantly decreased by DE, GI and even by pre-treatment with DE in GI group (p < 0,01). However, HA level was non-significantly (ns) decreased by DE, while significantly decreased in GI and DE – pretreatment to GI subjects (p < 0,01).

**Calcium, sodium and potassium variations (table 2):** Ca was significantly decreased by DE (p <0,01), but significantly increased by GI (p < 0,01), and pretreatment to GI with DE also significantly decreased Ca level (p < 0,01). Na level was ns increased by DE and by GI, but significantly increased by pretreatment with DE prior to GI (p < 0,01). K level was ns increased by DE and DE prior to GI and decreased ns by GI alone.

**Discussion**

**Biogenic amine variations:** The present study demonstrated that whole body GI significantly decreased NA blood level. However, DE significantly elevated NA level if taken alone and when given before GI it significantly prevented NA decrease. It was reported that disturbed levels of neurotransmitters like NA, DA and their oxidation metabolites play a pivotal role in neurodegenerative diseases [24]. The significant decrease in DA level by GI which was corrected by DE may participate in part in predicting a corrective role of DE for GI-induced depression and motor abnormalities due to NA vibrations, taking into account that DA is a precursor of NA [4]. This decreased blood DA level is probably due to a compensatory mechanism as DA content in rat brain basal ganglia was noticed to be increased after whole – body GI in rats [25]. 5-HT was significantly decreased in all groups relative to control. DE could not restore 5-HT level near to normal. It was stated that there is serotonergic dysfunction in cases of major depression accompanied by an over all alteration in 5-HT turnover rate [26]. Collectively, neurotransmitter concentrations in cerebral regions reflect the status of endogenous depression. Thus, NA, DA and 5-HT systems are disturbed in cases of depression. Remodulation of the level of these biogenic amines seems to be in charge of alleviating the etiology and hence the symptoms of depression [27]. The role of histamine in the control [28] and diagnosis of tumors [29] was reported before, which directed the interest to study blood histamine levels in GI subjects. Also, to find out its possible variations under DE treatment to follow up its contribution in radio- or herbal therapy with DE.

From the present results, it is clear that GI significantly lowered HA blood level and DE couldn't change this action and only DE alone non-significantly decreased HA level. These results coincide with a previous report showing that GI on the stomach might reduce gastric histamine without inducing a general histamine release [30].
Table 1. Serum levels of NA, DA, 5-HT and HA in DE – treated, GI and DE + GI – rats, compared to control. Values are represented as M ± SE, (n=10).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group</th>
<th>NA (µg/mL)</th>
<th>DA (µg/mL)</th>
<th>5-HT (µg/mL)</th>
<th>HA (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>163.2 ± 2.1</td>
<td>0.78 ± 0.009</td>
<td>0.31 ± 0.003</td>
<td>0.022 ± 0.0002</td>
<td></td>
</tr>
<tr>
<td>DE</td>
<td>187.4 ±1.5 **</td>
<td>0.9 ±0.006 **</td>
<td>0.052 ± 0.0004**</td>
<td>0.021 ± 0.0002</td>
<td></td>
</tr>
<tr>
<td>GI</td>
<td>81.2 ±1.4 ***</td>
<td>0.73 ±0.007 **</td>
<td>0.046 ± 0.001 **</td>
<td>0.02 ± 0.0004**</td>
<td></td>
</tr>
<tr>
<td>DE before GI</td>
<td>162.6 ±1.94</td>
<td>0.75 ± 0.009 *</td>
<td>0.044 ±0.002 **</td>
<td>0.02 ± 0.0004**</td>
<td></td>
</tr>
</tbody>
</table>

* Significantly different from control at p < 0.05.
** Significantly different from control at p < 0.01.
*** Significantly different from control at p < 0.001.

Table 2. Serum levels of Ca, Na and K in DE – treated, GI and DE + GI – rats, compared to control. Values are represented as M ± SE, (n=10).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group</th>
<th>Ca (mg/dL)</th>
<th>Na (meq/L)</th>
<th>K (meq/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3.54 ± 0.16</td>
<td>0.17±0.005</td>
<td>0.073 ± 0.001</td>
<td></td>
</tr>
<tr>
<td>DE</td>
<td>1.96 ± 0.016 **</td>
<td>0.19±0.008</td>
<td>0.079 ± 0.002</td>
<td></td>
</tr>
<tr>
<td>GI</td>
<td>4.2 ± 0.12 **</td>
<td>0.18±0.008</td>
<td>0.07 ± 0.005</td>
<td></td>
</tr>
<tr>
<td>DE before GI</td>
<td>2.7 ±0.12 **</td>
<td>0.21±0.008**</td>
<td>0.077 ± 0.002</td>
<td></td>
</tr>
</tbody>
</table>

* Significantly different from control at p < 0.01.

Calcium, sodium and potassium variations: In the nervous system, intracellular Ca activates K channels which in turn regulate the membrane excitability of neurons and play a major role in the neurotransmitter release. This contributes to both neuronal signal encoding and integration [31]. From these considerations we studied Ca, Na and K levels in combination with biogenic amines (neurotransmitters) to outline the possible pathways included in mood disturbances induced by GI and to introduce a better understanding for the possible role of DE in amelioration of these variations. This is because presence of both Ca and Na in drinking water was reported to activate and improve post-radiational activity and regeneration processes in GI rats [19]. In the present work, GI significantly elevated serum Ca level with ns reduction in serum K and rise in Na level. This increase in Ca level in the serum may be due to its decreased cellular influx, which probably reflects the significant decrease in all studied neurotransmitter blood levels. Pre-treatment with DE corrected Ca level near to normal with significant elevation of Na and ns elevation of K levels. This action is associated with a significant correction of NA levels if compared to GI group. Nevertheless, this action was accompanied by significant lowering of DA, 5-HT and HA levels. The role of DE is probably more accepted when considering NA, DA and K levels in DE group. Thus, Ca serum level when decreased, both Na and K levels were elevated, which may contribute to more intracellular Ca availability and K channel activation, with consequent elevation of NA and DA levels. This latter action could be supported by the suggestion that Ca triggers exocytosis of neurotransmitters or hormone-filled vesicles, which is considered as the main mechanism for cell to cell-communication in animals [32].

From these results, we could suggest that GI basically induces decrease of intracellular Ca availability with consequent decrease in K-channel activation, leading to decreased NA, DA, 5-HT and HA release. DE when given alone or before GI participated clearly in reversing this pathway, leading ultimately to normalization of serum NA level and correcting DA level than GI subjects. The concomitant decrease in serum calcium with elevated NA and DA levels in DE-treated group potentiate the recommendation of using DE as a radio protective pre-medication for neurotransmitter (specially NA) - related GI-accompanied sequela. Further clinical studies on the use of alcoholic extract of Damsissa in clinical practice may have promising medical utilities.

References


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