Preclinical evaluation of carnosine and Costus as hematological protective agents against gamma radiation

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Abstract

Background: Carnosine is an antioxidant dipeptide and Costus speciosus is an Indian herb with powerful antioxidant activity.

Purpose: Study the possibility of using Costus and carnosine to treat hemolysis concerns induced oxidative stress by irradiation.

Materials and methods: A total of 28 adult male albino rats were divided into four groups. First sham control group, and in the other three groups, their whole bodies were exposed to gamma irradiation with a single 7.5 Gy dose. The third and fourth groups were injection with the extract of Costus speciosus and carnosine, respectively. At the end of the experiment (on the 3rd day after irradiation) hematological parameters and related biochemical parameters such as erythrocytic LDH, G6PD, direct and indirect bilirubin were estimated.

Results: Costus significantly protects all hematological parameters such as hemoglobin, RBCs, reticulocytes, hematocrit and WBCs against hemolysis-induced radiation due to its powerful antioxidant effects. Also Costus retained LDH, G6PD, and bilirubin levels to normal levels even with the exposure to irradiation. Carnosine improved all the previous hematological and biochemical parameters from γ-irradiation but carnosine showed less potency than Costus.

Conclusions: Carnosine and Costus as antioxidants can protect against γ-radiation exposure and Costus speciosus was more potent than carnosine concerning hemolysis-induced gamma radiation.

1. Introduction

Hematological concerns are the most important issues in human life. One of the major aspects of blood, RBCs and Hb relations, which confers gas transport metabolism, buffering action, and toxic metabolite primary target instead of longer life structural tissues such as glycation by forming HbA1c, inhibiting formation of tissue advanced glycation endproducts (Klein & Anstee, 2008). Blood indirectly protects other tissues against excessive oxidative stress, because RBCs with relative short life acting as a free radical scavenger protect structural proteins with longer life span (Valko et al., 2007).

The protection of RBCs and Hb aids the body to maintain its physiological function. Accordingly, oxidative stress due to hematological concerns interferes with RBCs' integrity such as diseases (favism), bacteremia and viral infections, or even accidents (Valko, Morris, & Cronin, 2005). Many drugs enhance oxidative stress-induced anemia such as antineoplastics, furosemide (common diuretics), and nizatidine (antiulcer) etc., and genetic factors predispose oxidative stress-induced anemia such as favism or exaggerate drug hypersensitivity (Valko et al., 2007).

Many drugs and diseases may cause oxidative stress conditions mostly leading to thrombocytopenia and leukocytopenia such as renal failure, bacteremia, shock, and fever (Valko et al., 2005). We should also note that brain has some structural similarities to the blood in two ways. Brain has high rate of glycolysis and energy consumption. Brain constitutes 2% of body weight and consumes 20% of oxygen. RBCs produce less number of ATP in its glycolytic pathway leading to extra need of glycolysis activation. Microglial cells, which constitute up to 15% of brain cells, are macrophage in origin, which may relate blood diseases to brain concerns (Tomlinson & Gardiner, 2008). So treatment by antioxidants (such as carnosine and Costus) may alleviate such conditions.

Costus is a magical medical herb that has powerful antioxidant activity inhibiting inflammatory mediators with powerful antioxidative action also acts as antihistaminic, anti diabetic, and anticancer. Costus modulates the immune system via decreasing leukocyte count and enhances intracellular adhesion factor ICAM-1 (Anyasor, Funmilayo, Odutola, Olugbenga, & Oboutor, 2014). Costus has many pharmacological properties such as antioxidant, anticancer, anti-inflammatory, anti diabetic, hypolipidemic, hepatoprotective, steroidogenic, adaptogenic, and antimicrobial effects (El-Far et al., 2018).
Carnosine is an endogenous antioxidant with powerful free radical scavenging activity and it is sequester-free iron and captures iron-catalyzed oxidative stress alleviating such hematological concerns. Both carnosine and Costus are well known antimutagens with high margin of safety, thus so no further studies are needed for toxicity and teratogenicity (Anyasar et al., 2014). Costus is an antioxidant widely used as medicinal herb with no reported adverse effects on humans and in the animal studies (Medagama and Bandara 2014 ; El-Far et al., 2018). Carnosine showed no side effects being an endogenous anti-senescence agent (Gallant, Semyonova, and Yuneva, 2000).

Many components were isolated from *C. speciosus* by phytochemical screening and have a wide variety of biological activities, including alkaloids, glycosides, steroids, phenolic flavonoids, polyphenols, tannins, β-carotene, diosgenin, β-sitosterol, furostanolic saponins, costusosides, β-D-glucoside, prosapogenins, dioscin, gracilin, dihydroxyphytlplastoquinone, and α-tocopherolquinone. Moreover, β-amyrin, camphene, costunolide, diosgenin, α-Humulene, lupeol, and zerumbone for anticancer activity were recognized (El-Far et al., 2018).

Carnosine is a dipeptide (beta-alanyl-L-histidine), a non-enzymatic free radical scavenger, and a natural antioxidant. It may exert antioxidant activity by inhibiting lipid oxidation, including the oxidation of LDL and by free radical scavenging (Mocchegiani & Straub, 2004).

The higher radiation dose the more expressive extent and depth of changes occurred, so we used 7.5 Gy gamma radiation for treatment. Also it has been reported a stable hematological response after 3rd day of irradiation treatment by 7.5 Gy and irradiation treatment with radio protector as reported (Mackova, Lenikova, Fedorocko, Brezani, and Fedorockova 1996).

We should note that little or no attempts used irradiation as blood disorder simulator or used carnosine and Costus to improve blood disorders. All of the above blood concerns resulted in redox imbalance conditions encourage us to simulate such oxidative stress concerns via irradiation to explore some hematological troubles with respect treatment by antioxidants (such as carnosine and Costus) as a preclinical treatment evaluation study.

2. Materials and methods

2.1. Chemical preparations

2.1.1. Costus preparations

50 g of Indian Costus root powder (*C. speciosus*) was extracted by maceration in 500 ml hot water then set aside to cool for 2 h followed by filtration using Whatmann no 1 filter. The filtrate was lyophilized and collected. The resulting powder was weighed and dissolved in saline (100 mg/ml) and filtered through Millipore bacterial filter and collected in 10-ml vial (Singh & Maceration, 2008).

2.2. Carnosine preparation

Carnosine was purchased from Fluka Chemicals Company, Sigma Aldrich, packed in Switzerland. Carnosine was dissolved in saline (100 mg/ml).

2.3. Irradiation and animal protocol

All the experiments used laboratory animals followed the criteria of the investigation and ethics committee of the community laws enrolling laboratory animals. Irradiation was performed using Canadian Gamma cell-40(137 Cs) at Egyptian atomic energy authority. Biochemical studies were processed in Zagazig National Research Center, Faculty of Medicine, Zagazig University. Hematological analysis was performed in Royal Lab, Cairo.

Twenty eight male Sprague dawley rats weighing about 150–200g were classified into four groups. Group A, which is sham operated through i.p. injection of saline as a negative control group while Group B was injected i.p. with saline by 1 h prior to 7.5 Gy irradiation as positive control group. Rats in Costus-treated group were injected i.p. with a dose equivalent to 375 mg/kg of *C. speciosus* 1 h before 7.5 Gy gamma irradiation (Ezeijiofor, Orish, Orisakwe, & Gawl, 2014). Rats in carnosine- treated group were injected i.p. with a dose equivalent to 200 mg/kg of carnosine 1 h before 7.5 Gy gamma irradiation (Awwad, Abd El-Azim, Marzouk, El-Ghany, & Barakat, 2011). We thought that we don’t need to make isolated groups of Costus and carnosine because many other authors proved previously that they are strong antioxidants without side effects as mentioned in our paper. So we tried to know if they have antidiabetic and radio-protective effect against gamma radiation.

All groups were decapitated on third day after irradiation (Mackova et al., 1996), after anesthetization of the rats by i.p. administration of 90 mg/kg ketamine and 10 mg/kg xylazine (Meryem, Kanter, & Uzal, 2009). The biological effects of these anesthetics were considered to be negligible for two approaches. The first approach is that these pharmaceutical compounds were injected few minutes prior to decapitation, and the second approach is the injection of these compounds to all groups including control that was sham-operated one. Trunk blood samples were collected into two sets of EDTA-coated tubes. The first set is intended for hematological analysis while the second oriented toward spectrophotometric analysis. RBCs, reticulocytic count, leukocytic count, platelets, hematocrit, and Hb concentration were estimated by blood cell counter (Cell dine 1700).

Glucose-6-phosphate dehydrogenase was estimated by measuring the absorbance change at 340 nm as a result of glucose6-p with NADP+ to yield gluconate –6-P + NADPH+H+ (Kornberg et al., 1955).
Estimation of total and indirect bilirubin depends on reaction between bilirubin and diazonium salt of sulfanilic acid forming azobilirubin in presence of DMSO as total bilirubin and in absence of DMSO to estimate conjugated bilirubin (Walter & Gerade, 1970).

LDH was determined in RBCs with kits (Boehringer Mannheim, GmbH, Mannheim, Germany). The determination of LDH activity with the kit was based on the formation of diformazan by reduction of nitroblue tetrazolium in a reaction catalyzed by diaphorase with NADH. NADH was formed from NAD used as a cofactor catalyzed by LDH. Absorbance at 560 nm was measured with a spectrophotometer (U-2000; Hitachi Ltd.) (Shenawy, Nahla, Soliman, & Reyad, 2008).

### 2.3.1. Statistical analysis

The statistical package for social sciences SPSS/PC computer program was used for statistical analysis of the results. Data were analyzed using one-way analysis of variance (ANOVA) followed by Newman–Keuls post hoc test for multiple comparisons. The data were expressed as mean ±S.E. Differences were considered statistically significant at (P < 0.05).

Values in the same raw with different letters (a, b, or c) are significantly different. Means followed by the same letter (a, b, or c) are not significantly different.

The study was approved by the Animal Ethics Committee and in accordance with the guidelines set out by the Egyptian Atomic Energy Authority.

### 3. Results

Data of Table 1 revealed that RBCs, hemoglobin, white blood corpuscles reticulocytes, platelets, HCT, and MCHC were significantly decreased in γ-irradiated group compared to control group, and then improvement was noticed in treated groups with Costus and carnosine.

Results of Table 2 show that erythrocytic LDH, total bilirubin, direct and indirect bilirubin were significantly increased in γ-irradiated group compared to control group. On the other hand, G6PD was significantly decreased in γ-irradiated group compared to control group. The results of hematological and biochemical parameters showed hemolysis protection from both carnosine and Costus against gamma radiation with superiority of Costus.

### 4. Discussion

As the life advances, the radiation exposure consequences increase due to increase in nuclear aspects such as nuclear accidents and extensive use of therapeutic agents. Radiation exposure induces change in metabolic concerns altering the normal redox balance. By the change in redox balance, the metabolic-free radicle will increase, leading to increase in oxidative metabolites such as oxidized lipids, protein, and DNA. These products are produced as a result of increase in tissue damage. Such damage reflects the damage of underlying organelles.
causing cellular function disruption or even cellular death (Ghaemi et al., 2016).

The mostly affected organs by oxidative stress depend on mobility kinetics, metabolic rate, and growth of such organs with oxidants. Blood is a unique organ for such mobility being exclusive mobile carrier between different organs. Also blood is like an organ with high metabolic rate due to RBCs’ integrity that depends on Na/K ATPase energy-consuming reactions. Also high metabolic rate of blood conversion of glucose to lactate without intervention of tricarboxylic acid cycle due to absence of mitochondria. Blood is sensitive to oxidative stress as a consequence of its precursors that have high replication rate resulting in high metabolic rate causing their liability to oxidative stress-induced hematological malformation. Also the primary acceptor for such oxidants protects other tissues (Turker & Yel, 2014).

The above aspects associated with quite similar profile. Hematological concerns induced oxidative stress such as irradiation, favism, infection, and drug toxicity. Such similar profiles guided us to search hematologically induced oxidative stress condition by irradiation simulation for such diseases with the possible treatment. By application of radiation to Sprague dawely, their hematological profiles showed decrease in most values. Erythrocytic concerns significant decrease such as hemoglobin, hematocrit, and erythrocytic count as a result of bone marrow depression, hemolysis, and loss of some blood through microangiopathic hemorrhage (Derakshankhah et al., 2016).

Rat irradiation showed marked inhibition hematopoietic tissues, which in turn decreased erythropoiesis and blood formation. Disturbance of hematological indices may assign to disturbance in erythropoiesis (Shanmugam et al., 2015). C. speciosus-treated group showed significant improvement of hematologic concerns. Some studies found a higher erythrocytes’ anti-oxidant potential indicated by the marked decline in malondialdehyde (MDA) and the improvement of TAC in C. speciosus-supplemented animals (El-Far & Abou-Ghanemna, 2013), which supports our findings of providing protection to hematologic parameters. Moreover, the antioxidant effect of C. speciosus is due to the presence of antioxidant molecules such as ascorbic acid, β-carotene, α-tocopherol, glutathione, phenolic, and flavonoids (Devi & Urooj, 2010).

Carnosine showed protection to the erythrocyte against radiation. Results are in agreement with Nagai et al. who found that the red blood cell count, hematocrit, and hemoglobin level were also increased by the administration of carnosine, suggesting a protective effect of the agent against hemolytic anemia, and membrane stabilization is considered to be the mechanism of this effect (Nagai, Suda, Kawasaki, & Yamaguchi, 1990).

Costus extract showed more efficient protection than carnosine which may be through its powerful antioxidative effect. Also the superiority of Costus may originate from its higher lipophilicity than carnosine, which delays its urinary excretion and improves cellular membrane absorption. Both Costus and carnosine alleviate erythrocyte-induced oxidative stress, which is indicative of possible use of such agents as preclinical trials for favism and blood toxicity. Vijay-Kumar stated that administration of antioxidants enhances bone marrow cellular replication, protection, and differentiation through apoptosis modulation (Vijay-Kumar et al., 2008).

Reticulocytes percent is an indicator for hemolysis and erythropoiesis process. Dubner et al. found that irradiation causes initial decrease in reticulocytes percent below the normal level in the first week after irradiation followed by increase in the second week because of initial depression of erythropoiesis by 2Gy irradiation (Dubner et al., 1996). Our results showed that marked decrease in reticulocytes percent by irradiation compared to sham control group due to marked depression in erythropoiesis process. Carnosine-treated group showed significant decrease in reticulocytes percent in comparison to sham control group with significant improvement than positive irradiated control group due to its moderate protective effect. Costus-treated group showed non-significant increase in reticulocytes percent in comparison to sham control group while Costus-treated group showed high significant increase in reticulocytes count in comparison to irradiated positive control group due to its powerful healing effect, which enhanced the erythropoiesis process. By combining the results of bilirubin and reticulocyte percent, we can conclude that moderate hemolysis in Costus-irradiated group was compensated by prompt regaining of erythropoiesis ability.

The data of the present work elucidated that platelet count was most significantly ameliorated in comparison to irradiated group and carnosine-treated group. The Costus-treated group was highly protected by comparing its results with that of negative control group even in oxidative stress conditions, which is compatible with data of Uwah, Ewere, and Ndem (2015).

Leukocytic count showed significant change as a result of radiation exposure both Costus and carnosine alleviate such change as being powerful radioprotective agents (Wu et al., 2013; Aggarwal et al., 2006). The oxidative stress-induced irradiation or similar consequences have been associated with lymphopenia, neutropenia, thrombocytopenia, and anemia. The irradiation may disturb immunity and blood function. Thus, costus protected against blood and immunity dysfunction beside its anti-infection effects (Ojelere, 2014). Anti-leukopenic effect of carnosine was also
recorded by Nagai et al. (1990) in gamma-irradiated rats. But Costus has numerous advantages over radiation and hemoprotection. It can protect against harmful effects produced by cytotoxic drugs such as antineoplastics.

Bilirubin and glucose-6-phosphate dehydrogenase by irradiation exposure showed quite similar profile to favism and blood toxicity profiles, which indicate the possible use of irradiation as simulation for such troubles (Dybing et al., 2002). Bilirubin is a diagnostic marker for blood hemolysis. Our finding showed marked increase in both conjugated and non-conjugated bilirubin post 7.5 Gy of gamma irradiation exposure. These results augment the fact of hemolysis-induced irradiation. The non-conjugated and conjugated bilirubin are markedly increased with higher level of significance than control, which indicates rapid hemolysis with decreased liver ability to detoxify bilirubin by glycosylation through irradiation-induced hepatotoxicity. The rat treated with Costus showed significant decrease in bilirubin level in relation to irradiated rats whither conjugated or free bilirubin which indicates potent blood protection with hepatoprotective effect of Costus (Seif, 2016). Carnosine-treated group significantly differs from both non-irradiated control and irradiated control rats, because carnosine has strong antioxidant properties but not enough to regain bilirubin to normal level. Also Costus decreased bilirubin level than carnosine regarding total and indirect bilirubin. This reflects Costus alone that has more powerful antioxidant effect than carnosine (Zainuddin et al., 2018).

Also the enzyme Glucose-6-phosphate dehydrogenase was greatly suppressed (like favism) by gamma irradiation. The inhibition of Glucose-6-phosphate dehydrogenase reflects inhibition of blood reducing power. The reducing power of the blood represented in reduced glutathione as a result of NADPH production by the action of Glucose-6-phosphate dehydrogenase (Goel, 2014). Costus alleviates inhibition of blood reducing power by protection of Glucose-6-phosphate dehydrogenase enzyme and its required media. Carnosine also significantly alleviated inhibition of Glucose-6-phosphate dehydrogenase by but still less potent than C. speciosus. The more powerful effect of Costus may be attributed to its antioxidant properties and its higher lipophilicity attributed to cellular absorption by its high ability to penetrate lipid cellular membranes. Also its lipophilic terpenoids may confer lower clearance from erythrocytes intracellular compartments. Also C. speciosus may have the ability to stimulate tissue repair system.

Erythrocyte lactate dehydrogenase LDH showed marked increase as a result of intracellular release of LDH due to rat cellular destruction. Such cellular destruction may be attributed to destruction of cellular membranes due to formation of oxidized polyunsaturated fatty acids PUFA (Hamza & Al-Harbi, 2015). LDH enzyme of anaerobic glycolysis, which reversibly converts pyruvate forming lactate in RBCs and tissues lack of ability for completing oxidation processes. Irradiation was found to increase LDH activity as a result of release of intracellular LDH to the general circulation. Costus extract showed high significant alleviation by reverting the LDH to normal level due to powerful protecting effect of C. speciosus. Also contains lipophilic isoprenes, which can interact with cellular membrane lipid offer membranous powerful protection (Seif, 2016). While carnosine offered significant change in LDH profile compared to irradiated and non-irradiated control rat groups. This elucidate Costus is powerful anti-hemolytic radio protector than carnosine (Babizhayev, 2014).

In conclusion, all the above data augment the effect of carnosine and Costus against hematological and biochemical changes due to ionizing radiation. Costus and carnosine improve hematological troubles of irradiation redox imbalance conditions, which simulate hematological oxidative stress concerns (such as favism and hemotoxicity) with respect to treatment by antioxidants as a preclinical treatment evaluation study.

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No potential conflict of interest was reported by the authors.

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