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Original Research Article

Radioprotective and anti-diabetic effects of *Costus speciosus* and carnosine

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Abstract

Purpose: *To evaluate the possible radioprotective effect of Costus speciosus and carnosine as natural antioxidants in order to control the hyperglycemia developed in male albino rats exposed to acute oxidative stress induced by gamma radiation.*
Methods: *Twenty-eight adult male albino rats were divided into four groups. The first group was taken as a control group, while the three other groups were exposed to ɣ irradiation at a single 7.5 Gy dose. Furthermore, the rats in the second and third groups were i.p. injected with Costus speciosus root powder and carnosine, respectively. On the 3rd day, after irradiation, the serum levels of glucose, insulin, C peptide, copper, iron, calcium, total antioxidant capacity (TAC) and malondialdehyde (MDA) were measureded.*
Results: *The results revealed that exposure to ɣ irradiation induced significant increases in serum glucose, iron, and malondialdehyde. However, the levels of serum calcium, copper, total antioxidant capacity and insulin significantly decreased ($p < 0.05$). A significant decrease was observed in C peptide in the exposed group, compared to control group. All the test parameters indicate improvement after treatment with Costus speciosus and carnosine ($p < 0.05$).*
Conclusion: *Costus speciosus and carnosine ameliorate the effect of gamma radiation, indicating their role as antidiabetic agents and radioprotectors; however, Costus speciosus was critically more efficient than carnosine.*

Keywords: *Costus speciosus, Carnosine, Diabetes, Insulin, Gamma radiation protection*

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INTRODUCTION

Over a long period of time, much interest has been devoted to the management of diabetic disorders in order to find a selected folk remedy with a curative impact on diabetes mellitus. The trials followed different lines of research including chemically discovered drugs, medicinal herbal remedy such as all costus species, vitamins and antioxidants like carnosine as antioxidant chelating agents [1].

Among costus species, Costus speciosus is an Indian plant characterized by its potent antioxidant properties. In *C. speciosus*-supplemented animals, higher erythrocytic antioxidant potential was improved [2]**.** All Costus

species have modulating effects upon inflammatory mediators related to development of insulin resistance including cytokines and interlukin6; besides leptin as a proinflammatory protein and adiponectin as anti-inflammatory protein [3] *C. speciosus* has a wide variety of biologically active components including alkaloids, glycosides, steroids, phenolic chelating agents, β-carotene, , β-D-glucoside, etc. [4]**.**

However, it has been postulated that the improvement of glucose metabolism induced by costus species is related to proinsulin conversions which promote this process [3]. Thus, costus does not only modulate the immune affected cells, but also brings about possible healing and regenerating effects. Moreover, most of costus species showed potential healing and cellular antiaging effects revealing tissue regeneration [5].

Therefore, this study is an attempt to find cheaper and safer radio-protective herbal antioxidants with more potent and less harmful effects than those produced by synthetic drugs.

Carnosine is considered one of the most powerful chelating agents. It is a dipeptide antioxidant preventing diabetic deteriorations through antiapoptotic maintenance of immune cells inhibiting immunocompromised induced diabetic effects. Carnosine does not only modulate cellular immunity, but also regulates inflammation mediators in diabetic animals [5]. Carnosine has cellular protective effects, with expected delaying pancreatic deterioration and possible enhancement of insulin tissue resistance [6].

Gamma radiation is used as a simulator of chemically-induced hyperglycemia. The higher radiation dose produces more evident extent and depth of metabolic effects (hyperglycemia, accumulation of glycogen). Blood glucose and liver glycogen may serve as reliable and dose-dependent biological indicators of metabolic changes in the irradiated rats [7]. The 7.5Gy single dose radiation was chosen to induce hyperglycemia to study the radioprotective and antidiabetic effects of antioxidants.

For all the above-mentioned benefits of *Costus speciosus* and carnosine, they were selected to control hyperglycemia developed in the rats exposed to acute oxidative stress via gamma radiation. Besides, a comparison was conducted between the two agents under investigation to elucidate their efficiency as radioprotectors.

EXPERIMENTAL

Animals and management

Twenty-eight adult male albino rats (Sprague dawley) weighing 180 – 200 g were used in this study. They were bred and kept under the same living and nourishment conditions. All animals were collectively housed in polypropylene cages. The housing room was maintained at 24 °C with 42 ± 5 % relative humidity and under pathogen-free conditions. The animals were synchronized to a 12 – 12-h light–dark cycle with free access to water and feed.

Anesthetic procedures and handling of animals were approved and complied with guidelines of Medical Ethical Committee of the National Research Centre in Egypt (approval no. 14077), according to ethical guidelines for care and use of laboratory animals [8].

Costus preparations

Fifty grams of Indian costus root powder (Costus speciosus; family: Costaceae) extract (100mg/ml) was prepared according to Marzook *et al* [9]**.**

Carnosine preparation

Carnosine was purchased from Fluka Company. Carnosine and dissolved in saline (100mg/ml).

Experiment design and irradiation protocol

Adult male albino rats were obtained from the Animal Breeding Unit of Biological Applications Department (NRC, EAEA). The rats were randomly divided, on the basis of body weight, into four equal groups (seven per each). The studied groups were distributed as follows:

Group 1 (Negative Control): injected intraperitoneally (i.p) with a saline without gamma irradiation exposure.

Group 2 (Positive Control): one hour before gamma irradiation, i.p was injected with saline.

Group 3 (Exposed + Costus): one hour before 6 Gy gamma irradiation, i.p was injected with C speciosus equivalent to 375 mg/kg**.**

Group 4 (Exposed + Carnosine): one hour before gamma irradiation, i.p was injected with carnosine equivalent to 200 mg/kg by.

After anesthetization of the rats by i.p. administration of 90 mg/kg ketamine and 10

mg/kg xylazine, the last three groups were ɣ-irradiated with a single dose of 7.5 Gy. Whole body irradiation was performed using ^{60}Co -ɣ cell. The irradiation of animals was carried out in the National Center for Radiation Research and Technology, Cairo, Egypt. The Control rats (Group A) were similarly treated and taken to the theater, but they were not irradiated.

All groups were decapitated on the third day after irradiation**,** and trunk blood samples were collected. Sera were separated and frozen for subsequent analysis. Total Antioxidant Capacity was assessed according to the method of Koracevic *et al* [10] using an available commercial kit (Biodiagnostic Company, Giza, Egypt). Colorimetric determination of serum malondialdehyde (MDA) based on a commercial available kit (Biodiagnostic Company) was carried out to determine the lipid peroxide products. Total calcium was estimated according to the method of Gitellman [11]**.** Copper and iron were assayed by atomic absorption/flame-emissions spectrophotometer (Buck210VGP) using acetylene flame and hollow cathode lamp. Serum C-peptide was estimated using radioimmunoassay (Medgenix Diagnostics)**.**

Statistical analysis

Data are presented as mean± SE and analyzed by Student's T-test (Statgraphics, Origin6.1). Level of significance was expressed as p <0.01.

RESULTS

Table 1 summarizes the data for serum glucose, insulin, and C- peptide in the unexposed control, exposed untreated and exposed treated groups (carnosine and Costus speciosus).

The exposed untreated group showed a significant increase in serum glucose when compared to the healthy control and exposed treated (carnosine and *Costus speciosus*) groups, whereas the *Costus speciosus* Treated Group showed an insignificant difference when compared to the unexposed control group. However, serum glucose was significantly lower when compared to the carnosine treated group as made clear in Figure 1.

On the other hand, serum insulin was significantly decreased in the Exposed Untreated Group when compared to the Exposed Treated groups (carnosine and *Costus speciosus*) and the control unexposed group. Further, insulin showed a significant decrease in both the exposed treated groups (carnosine and *Costus speciosus*) as compared with the Control Group (Figure 2).

As for C peptide, no statistical changes were observed between the Control Group and the Exposed Treated groups (carnosine and Costus speciosus). The only significance in the C peptide level was recorded between the Control Group and the Exposed Untreated Group.

Serum iron showed an insignificant difference between the unexposed control and exposed treated groups (carnosine and *Costus speciosus*), whereas they experienced a significant decrease when compared to the exposed untreated group.

With respect to serum copper, it was significantly decreased in the exposed untreated and treated groups (carnosine and *Costus speciosus*) as compared to the unexposed control group. Nonetheless, it was significantly increased in the exposed treated groups (carnosine and *Costus speciosus*) as compared to the exposed untreated group. About serum calcium, the results denoted that it was significantly decreased in the exposed untreated group when compared to the unexposed control and exposed treated groups (carnosine and *Costus speciosus*), while it showed non-significant changes between the two exposed treated groups (carnosine and *Costus speciosus*) or the control group. MDA showed a significant increase of its level in the Exposed Untreated Group as compared to the Control Unexposed and Exposed Treated groups (carnosine and Costus speciosus).

Table 1: Serum glucose, insulin and C- peptide levels (mean ± SE)

Parameter	Negative control (unexposed)	Positive control (exposed)	Exposed+ carnosine	Exposed+ *Costus Speciosus*
Glucose (mg/dl)	126.71±5.03^{c}	214±7.54^{a}	164±5.7^{b}	134.71±8.11^{c}
Insulin (iu/ml)	48.29±1.62^{a}	18±0.62^{c}	37.57±1.13^{b}	38.14±0.71^{b}
C peptide (ng/ml)	0.133±0.014^{a}	0.086±0.0001^{b}	0.111±0.014 ^{a,b}	0.109±0.015 ^{a,b}

Different letters (a, b and c) in each row indicate significantly different values at p < 0.01**.** Data in Table 2 was expressed as mean ± standard error (X ± SE) for serum iron, copper, calcium, MDA and TAC in the unexposed control, exposed untreated and exposed treated groups (carnosine and *Costus speciosus*).

Meanwhile, it was significantly decreased in the Exposed costus Treated Group when compared to the Control Unexposed and the Exposed carnosine Treated groups (Figure 3).

As for TAC, it was significantly decreased in the Unexposed Control and Exposed Untreated groups when compared to the Exposed Treated groups (carnosine and costus speciosus). It was significantly higher in the Control Unexposed Group than the exposed untreated group. Nevertheless, it was significantly higher in the costus treated group than in the carnosine Treated Group as shown in Figure 4.

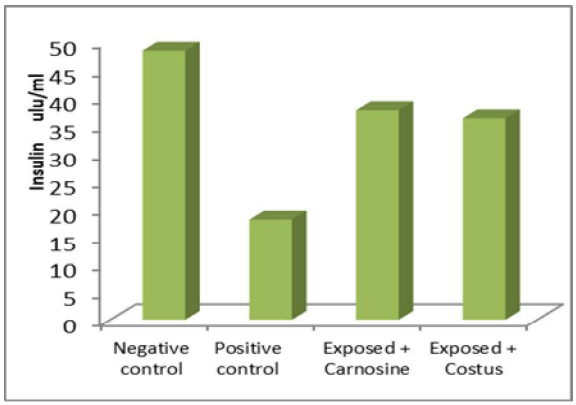

Figure 1: Serum glucose level in the studied groups

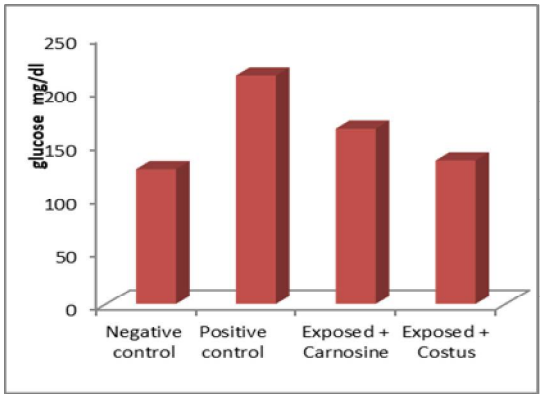

Figure 2: Serum insulin level in the studied groups

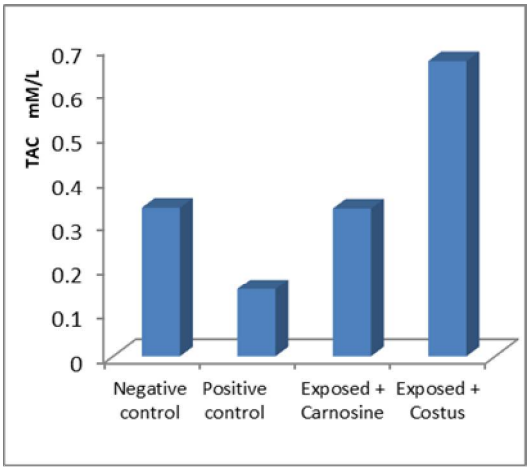

Figure 3: Serum MDA level in the studied groups

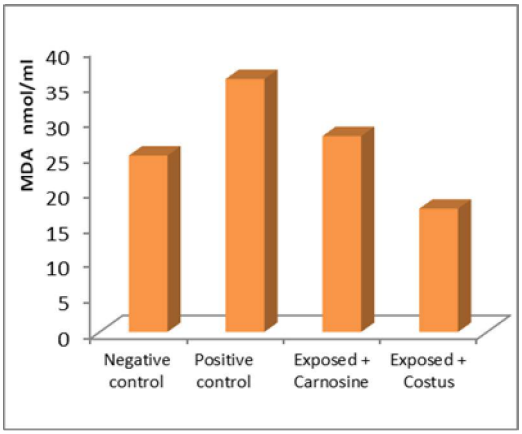

Figure 4: Serum TAC level in the studied groups

DISCUSSION

The goal of this study is to control hyperglycemia by using costus species as a means of herbal therapy and carnosine as a natural antioxidant and chelating agent to overcome oxidative stress induced by γ-radiation.

Table 2: Serum iron, copper, calcium, MDA and TAC levels

Parameter	Control negative control (unexposed)	Positive control (exposed)	Exposed+ carnosine	Exposed+ *Costus Speciosus*
Iron (µmol/l)	24.41±0.91[b]	35.61±2.01[a]	23.37±0.98[b]	21.71±1.85[b]
Copper (mg/dl)	186.5±5.71[a]	142.27±4.4[c]	164.94±3.08[b]	166.34±10.33[b]
Calcium (mg/dl)	11.97±0.32[a]	10.53±0.27[b]	12.29±0.35[a]	12.74±0.36[a]
MDA (nmol/ml)	25.04±1.15[b]	35.86±1.36[a]	27.76±1.73[b]	17.44±1.47[c]
TAC (mM/L)	0.336±0.012[b]	0.151±0.022[c]	0.334±0.057[b]	0.669±0.093[a]

Note: a, b and c in each row means that different letters are significantly different at $p < 0.01$

All previous studies encourage researchers to test the curative effect of Costus speciosus and carnosine against such disturbances which are mainly associated with oxidative stress in order to reveal a favorable remedy [12-15].

Oxidative stress, induced by irradiation, causes damage in the liver of rats in ferritin (the iron storage protein) degradation and increases in the intracellular free iron level. Since the ferritin level is proportional to tissue iron stores, it is considered one of the most sensitive measurements of iron status besides hemoglobin and packed cell volume [16]**.** However, the increase in the serum iron level recorded herein in the irradiated animals may have resulted from the leakage of iron from its macromolecular complexes ferritin and transferrin [14]**.** Hence, the significant decrease of serum copper and the remarkable increment of serum iron noted herein in the gamma-irradiated group were in consistence with the above-mentioned reports.

In this study, the treatment of the irradiated rats with carnosine and Costus resulted in a sensible improvement of serum Cu content as compared to the Exposed Group. Despite such an improvement, the level of serum Cu was still lower than that in the control group. This result could be attributed to the ability of carnosine to bind certain heavy metals such as copper (II) as reported by Kyriazis [17]**,** but carnosine metal ion chelation seems to play only a minor role during oxidative stress. The results of the current investigation showed that carnosine and costus species were effective in the reversal of the alterations induced in serum iron concentration as compared to the irradiated group. Obviously, they almost brought back serum iron level towards the control value. Similarly, a protection of erythrocytes, hemoglobin, ferritin, and iron transport protein (transferrin) against free radicles destruction and releasing of iron may be anticipated. Hence, carnosine can conserve blood iron hemostasis, which is in accordance with Kyriazis [17].

In the present study, gamma irradiation induced significant hyperglycemia, parallel with a significant increase of Malondialdehyde. This result is in agreement with Saada *et al* [18]. It has been postulated that oxidative stress, which was induced by gamma irradiation, led to an obvious decline in the antioxidant defense system and seemed to have a central role in the onset and development of diabetes mellitus and its complication in rats [19] since reactive oxygen species are related to the destruction of pancreatic β cells and associated with consequent impairment of insulin secretion [1]**.** This is exactly what occurred herein as significant hypoinsulinemia was noted in the irradiated rats.

In the present study, treatment of irradiated animals with either carnosine or costus species was accompanied by a marked decrease in the levels of MDA, blood sugar and a significant rise in serum insulin. However, a more significant reduction was evident in the *Costus* group. With regards to MDA, the result of this investigation could be compared with the findings reached by Tawfik and Salma [20] who reported that the carnosine treatment prior irradiation was associated with a reduction of the level of MDA through its antioxidant properties. MDA is considered one of the carnosine multiple functions. In other words, it is a scavenger of zinc and copper ions and toxic aldehydes (MDA). The use of carnosine or costus species caused a significant rise of serum insulin in the irradiated rats, but the elevations were not sufficient to reach its normal value in the control group. All these benefits sustain *C speciosus* and carnosine to be dietary supplements that can modulate the biochemical changes induced as a result of exposure to gamma rays.

Eliza *et al* reported that the oral administration of eremanthin (a compound isolated from Costus speciosus) significantly reduced the plasma glucose and remarkably increased the plasma insulin in the diabetic rats [21]. Furthermore, Daisy *et al* showed that the root crude extract of *C. speciosus* possessed hypoglycemic action [21]. All these findings indicated that the *C. speciosus* and its derivative retained a more favorable antioxidant capacity which came in line with the obtained results and is confirmed by the findings announced by Eliza *et al* [21].

In the present study, although the treatment of irradiated rats with carnosine or C speciosus yielded better outcomes upon the levels of blood sugar (insulin and MDA), the efficacy was not enough to restore the levels of the above-mentioned under-examination parameters to their normal values of the Control animals. A longer period of treatment may have been needed to reach reference values. Hence, it could be concluded that carnosine and C. Speciosus could be used as drugs by diabetic individuals.

In the current study, serum calcium was significantly decreased in the gamma irradiated group, and then improvement occurred after treatment with Costus speciosus and carnosine.

The decreased serum level of calcium can be explained by the effect of oxidative stress induced by gamma radiation causing Ca^{2+} influx into the cytoplasm from the extracellular environment, endoplasmic reticulum or sarcoplasmic reticulum (ER/SR) through the cell membrane and the ER/SR channels, respectively. Raising the Ca^{2+} cytoplasmic level causes Ca^{2+} influx into mitochondria and nuclei [14]**.** Therefore, it can be suggested that the cytoplasmic Ca^{2+} influx from the extracellular environment by oxidative stress induced by gamma radiations would be the cause of decreased serum level**.**. Ca^{2+} influx can be attributed to changes in cell permeability developed by irradiation.

Moreover, higher concentrations of hydrogen peroxide cause a sustained elevation of cytoplasmic Ca^{2+}, with the assumption that severe oxidative stress causes Ca^{2+} uptake by the cells from extracellular spaces [14]**.** Though treatment with Costus speciosus and carnosine gives rise to inhibition oxidants which cause cytoplasmic Ca^{2+} uptake from the extracellular environment, the ER/SR is stored through the InsP3-gated channels (inositol 1, 4, 5 triphosphate). Further, oxidants inhibit the Ca^{2+} efflux from the cytoplasm to extracellular spaces through ATPase pumps. Na^{+}/Ca^{2+} exchangers can also be inhibited or even reversed, causing excessive Ca^{2+} storage in the cytoplasm which may be reflected on its serum level [13].

In the present study, the TAC in the exposed group significantly decreased in the Gamma Irradiated Group compared to the control Group. This study assumes that the generation of cellular reactive oxygen species (ROS), by ionizing radiation, is a result of water radiolysis [22]**,** and then the radiation damage is caused by the excessive production of ROS consuming cellular antioxidants. This leads to oxidative stress and cellular damage [6]**.** Ahlersova *et al* have reported that the exposure to ionizing radiation leads to the depletion of these endogenous antioxidants that enhance the development of systemic diseases [23]. Therefore, the current investigation suggests that treatment with both antioxidants provides protection against the effect of oxidative stress by destroying free radicals and harmful molecules which keep the endogenous antioxidants enzymes safe so as to maintain total antioxidant capacity. The obtained results are in agreement with those findings announced by El-Desouky *et al* who stated that a significant depletion in the antioxidant system is accompanied by enhancement of lipid peroxides after whole body gamma irradiation [24]. The current results revealed higher TAC values in the groups treated with costus and carnosine. These data indicate direct reductive power of costus and carnosine or their ability to decrease body oxidants [24].

C-peptide is formed during the insulin genesis process in a clear cut relationship. Thus, higher C-peptide values may indicate activation of insulin secretion, and this fact has been confirmed in the current study as the level of C-peptide in the sera of the rats exposed to γ radiation was significantly lower than its value in the Control Unexposed Group. This obviously reflects the negative impact of radiation on the pancreatic insulin genesis. The obtained results showed a non-significant increase in the C-peptide level, whereas the results of insulin showed a significant increase in the Carnosine and Costus Treated groups. These results can be explained in the light of the fact that both antioxidants may decrease the catabolic disposal of insulin extending its half-life. Besides, these results can be explained by the activation of proteolysis disposal of C-peptide in order to remove the excessive amounts of C-peptide to utilize its amino acids contents for further protein synthesis. Additionally, these results and their explanations need more investigation using further experiments [25].

CONCLUSION

This study revealed that *Costus* and carnosine as cheaper and safer radio-protective natural antioxidants with more potent and less harmful effects can potentially be used as anti-oxidants in diabetic patients and as radioprotectors in chemotherapy patients. *Costus* produces more potent radioprotective and antidiabetic effects than carnosine.

DECLARATIONS

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Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities

pertaining to claims relating to the content of this article will be borne by the authors.

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