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COMPARATIVE EFFECT OF CAROTENOID COMPLEX FROM GOLDEN NEO-LIFE DYNAMITE (GNLD) AND CARROT EXTRACT ON SOME HAEMATOLOGICAL PARAMETERS OF HEALTHY WISTAR ALBINO RATS

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ABSTRACT

The hematological implication of oral administration of a commercially produced carotenoid complex (from Golden Neo-Life Dynamite, GNLD) and carotene from carrot extract was investigated in albino wistar rats. The rats were assigned to 4 groups of 6 rats each consisting of group 1(control group treated with distilled water), group 2 (treated with olive oil), group 3(treated with carrot extract + olive oil) and group 4(treated with GNLD Carotenoid complex + olive oil). The concentration of carrot extracted carotenoid and that of the GNLD carotenoid complex administered were 559mg/kg.The animals were sacrificed after 21 days of treatment and whole blood collected for analysis. The hematological parameters investigated included the % hemoglobin (Hb), red blood cell (RBC) count (mm^3), white blood cell count (mm^3) and packed cell volume (mm^3). At the end of the experiment, the blood was collected into separate labeled heparized vial for haematological analysis. From the result, there was a statistically significant increase ($P<0.05$) in the Hb of the group treated with carotenoid complex + olive oil (8.48 ±0.10) as well as the group treated with carrot extract + olive oil (8.68 ±0.19) when compared to the control (7.48 ±0.17).There was also a significant increase ($P<0.05$) in the white blood cell count of the group treated with commercially produced carotenoid complex + olive oil (12,750.00 ±339.12) and that with carrot extract + olive oil (12112.00 ± 309.25) when compared to the control (11825.00 ± 150.00). The results for groups 3 and 4 for the PCV, RBC and Platelets count were non-significantly raised ($p>0.05$) compared to their controls. These increases can be attributed to the important role played by carotenoids in the synthesis of Red cell protoprophyrin which is a component of the haem biosynthesis pathway. This makes beta – carotene a possible treatment for Erythropoietic protopophyria. The increase in WBC count points to the immunomodulatory potentials of beta –carotene in enhancing the immune system as well as its anti-carcinogenic activity.

KEYWORDS: Beta carotene, vitamin A, carotenoids, hematological indices.

INTRODUCTION

Carotenoids are a class of natural fat-soluble pigments found principally in plants, algae and photosynthetic bacteria where they perform a critical role in the photosynthetic process. They also occur in some non photosynthetic organisms and protect against damage by light and oxygen, although animals appear to be incapable of synthesizing carotenoids from their diet. Within animals, carotenoids provide bright colors; serve as antioxidants and a source of vitamin A activity (Ong and Tee 1992, Britton *et al*, 1995.)

Carotenoids are responsible for many of the red, orange and yellow hues of plant leaves, fruits and flowers as well as the color of birds, insects, fish and crustaceans (Pfander, 1992). Some familiar examples of substances containing carotenoids are the oranges, carrots, citrus fruits, peppers, tomatoes, the pink of flamingoes and salmon (Pfander, 1992). Some 600 different carotenoids are known to exist naturally (Ong and Tee, 1992), and new ones continue to be identified (Mercandant, 1999).

Diets rich in fruits and vegetables are recommended to maintain overall good health and to prevent chronic diseases. Chemical compounds in healthy diets may modulate different aspects of immunity. Especially for beta – carotene, several studies suggest that this carotenoids may stimulate the immune system (Hughes, 1999).Supplementation of diets with pure beta carotene stimulated lymphocytes proliferation in several human interaction studies (Kramer and Buri,1997, Ven *et al*, 1993). Lytic activity of natural killer cells were enhanced in elderly subjects on long term beta carotene supplementation (Santos, *et al*,1991) and after short term supplementation the percentage of the natural killer cells were significantly increased (Washon *et al* ,1991).

Results from some human studies have also shown improvement of measures of antioxidant activity (decreased copper induced LDL oxidation, decreased DNA strand breaks and oxidized pyrimidine base in lymphocytes, decreased serum lipid peroxide levels, decreased pentane breath, decreased serum malondialdehyde, increased red blood cell copper /zinc superoxide dismutases activity) in individuals receiving high intake of beta carotene (Santos *et al*, 1996).

Recently, other carotenoids including lycopene and lutein have received more attention, several studies reveal that a high intake of the carotenoids is associated with a reduced incidence of prostate and lung cancer (Giovannucci, 1999, Michand *et al*, 2000).A high intake of tomato and tomato-based products is also consistently associated with a low risk of cancer for a variety of anatomic sites (Giovannucci, 1999). Numerous potentially immunomodulating compounds are present in tomatoes with lycopene being the major phytochemical (Beecher, 1998).

Beta-carotene and some other carotenoids have demonstrated some immunomodulatory effects .In healthy male non-smokers, beta-carotene supplementation (15 mg/ day) was found to significantly increase the percentage of monocytes expressing the major histocompatibility complex class II molecule, to increase the expression of the adhesion molecule-I and leukocyte function associated antigen 3 and to increase *ex vivo* secretion of tumor necrosis factor (TNF)-alpha by blood monocytes (Benlich,1991).

Beta carotene supplement has also been found to enhance natural killer cell activity in elderly men (Santos *et al*, 1996) and also increase lymphocyte response to mitogens in healthy male cigarette smokers and to increase CD4 lymphocyte count in some subjects with AIDS (Tauvain and Vechia, 1999). It is thought that the possible immunomodulatory activity may be independent of the role of beta carotene as a precursor of retinol (Omenn et al, 1996). Beta carotene has been found to inhibit the growth of some

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malignant cells, including human prostate cancer cell *in vitro* (Lee *et al*, 1999).

Frieling *et al*, (2000) speculated that beta carotene may increase the cellular differentiation, down regulate epidermal growth factor receptors, reduce adenyl cyclase activity, enhance expression of gap junctional proteins and protect against oxidative damage. The ability of beta carotene to modulate the carcinogenic process, at least *in vitro*, may be due, in part to its conversion to retinoids (Omenn *et al*,1996). In this regard there is evidence that beta carotene may be converted to retinol and other related metabolites (e.g. retinoic acid) in human prostate cell lines.

In most developing countries, people do not really have an understanding of the role played by the carotenoids. It is generally known to be a precursor of vitamin A, but apart from acting as a vitamin A precursor it is equally essential in the body in fighting against diseases and infection. It has been observed generally that people do not really incorporate fruits and vegetables into their diet probably because they do not have an understanding of the nutritional value of fruits. Fruits and vegetables are the main sources of carotenoids, since they are not found in animals, the benefits of these findings is that it will provide experimentally documented evidence on the health benefits of carotenoids and cause the level of knowledge and understanding on the need to incorporate carotenoids in the diet of the general population. This will make people live healthier and longer lives. Also diseases like heart disease, cataracts, cancer, and macular degeneration will be minimized.

MATERIALS AND METHODS

The carrots used for this research were purchased from the Marian market in Calabar, Nigeria. They were chopped into smaller pieces with a knife and dried in an Astell-hearson oven to a constant weight at 50°C and then grounded into powder using an electric blender. The carotenoid in the ground carrot was extracted using a Soxhlet apparatus; 10g of the dried extract was dissolved in 40ml of olive oil, the olive oil serving as an excipient and administered to the wistar albino rats at a dose of 559mg/kg body weight. The commercial Carotenoid complex supplement was purchased from a GNLD distributor in Calabar. The concentration of carotenoid in each capsule was 900mg; this was dissolved in 40ml of olive oil and administered at an oral dose of 559mg/kg body weight.

Twenty four male wistar albino rats weighing between 150 and 200g used were divided into four groups of six rats each and kept in plastic cages. They were fed with rat pellets and water *ad libitum*. The experimental group and treatment included; group 1(control) , group 2(treated with olive oil), group 3(carrot extract with olive oil), group 4 (GNLD carotenoid complex with olive oil). The administration was done orally and lasted for twenty one days after which the rats were sacrificed by suffocation using chrloroform after an overnight fast. The blood obtained by cardiac puncture was stored in heparinized vials and used for analysis of hematological indices.

Red blood cell (RBC) count was done using the method of Osim *et al*, (2004) which involved a 1:200 dilution of blood with Haymen's fluid and then counting in a special counting chamber under the microscope.
Hemoglobin concentration was determined using Sahli's method as reported by Osim *et al*, (2004). The hemoglobin present in a sample of blood was converted to acid hematin by addition of 0.1 N HCL and matched against a non fading brown color standard.

The packed cell volume (PCV) was determined by the method of Osim *et al*, (2004). This was done by measuring the percentage volume of blood cells after centrifuging whole blood samples at 14,500g for 5 minutes so that the red cells become packed at the bottom of the capillary tube and plasma remains on top.

White blood cell (WBC) count was estimated from heparinized blood by the method of Dacie and Lewis, (1991).This involved microscopic visual identification and counting of white blood cells in Turk's fluid.

Statistical analysis
The values were expressed as means ± standard deviation of six determinations. They were analyzed using analysis of variance (ANOVA) and Student's t-test

Table 1: Haematological indices of wistar albino rats treated with carrot extracted carotenoid as well as carotenoid complex from GNLD

Group	Treatment	PCV%	Haemoglobin g/100ml of blood	RBC (mm^3)	WBC (mm^3)
1.	Distilled water	45.25±5.19	7.48 ± 0.171	5. 45 ± 0.059	11,825.00 ± 150.00
2.	Olive oil	47.75 ± 1.26	8.23 ± 0.126	5.20 ± 0.123	11,812.50± 308.81
3.	Carrot extract + Olive oil	54.25 ± 3.77	8.68 ± 0.189 $^{\alpha}$	5.21 ± 0.026	12,112.00 ± 309.25 $^{\alpha}$
4.	GNLD carotenoid complex + Olive oil	51.00 ± 1.63	8.48 ± 0.096 $^{\alpha}$	5.32 ± 0.089	12,750.00 ± 339.12 $^{\alpha}$

Values are means ± standard deviations of six determinations
$^{\alpha}$ – significant increase (P<0.05) compared to the controls (Groups 1&2)

RESULTS AND DISCUSSION

From Table 1, there was no significant increase (*P*<0.05) in PCV in the groups which received commercially produced carotenoid complex + olive oil (51.00 ± 1.63) and carrot extract + olive oil (54.25 ± 3.77) when compared to the control (45.25 ± 5.19). However the result for group 2 which received only olive oil (47.75 ± 1.26) was comparable to that of the control group, this shows that the olive oil was used for suspending the drug and had no significant effect on the immunomodulatory potentials of the carotenoid complex and carrot extract.

As shown in the table, there was a significant increase (P<0.05) in haemoglobin content in group 3 and 4 which received carrot extract + olive oil (8.68 ± 0.19) and commercially produced carotenoid complex + olive oil (8.48 ± 0.10) when compared to the control (7.48 ± 0.17). This significant increase makes carotenoids useful in increasing haemoglobin synthesis. Substances that increase haem synthesis have been used in the treatment of erythropoeitc protoporphyria (Hughes *et al*, 1997).Protoporphyrin synthesis occurs in the mitochondria which is converted to haem and finally incorporated to globin to form haemoglobin in the developing red blood cells.

There was no significant decrease (P>0.05) in the red blood cell count in group 3 and 4 which received carrot extract + olive oil (5.21 ± 0.026) and commercially produced carotenoid complex + olive oil (5.32 ± 0.09) when compared to the control (5.45 ± 0.06).Some human studies by Santos *et al*, (1996) indicated that there was an increased red blood cell copper / zinc superoxide dismutase activity in individuals receiving relatively high intakes of beta carotene but no changes or inconsistent changes in the red blood cell copper zinc superoxide dismutase activity for these subjects receiving relatively low levels of beta carotene.

There were significant increases in the white blood cell count in groups 3 and 4 which were treated with carrot extract + olive oil and commercially produced carotenoid complex + olive oil when compared to the control . The result for group 4 which was treated with only olive oil was comparable to that of the control. This significant increase in the WBC count is attributed to the immunomodulatory potentials of the carotene content of the carotenoid complex and the carrot extract .In the report of Bendich ,1991 on healthy, male non smokers, beta carotene supplementation (15mg/day) was found to significantly increase the WBC count (monocytes) and leukocytes function associated antigen-3 and tumor necrosis factor (TNF) alpha by blood monocytes .Also beta carotene supplementation has been found to increase lymphocytes response to mitogens in healthy male cigarette smokers and to increase the CD4 lymphocyte count in some subjects with AIDS although the mechanism of these immunomodulatory activity of beta carotene is not known. Also there was no significant increase (P>0.05) in the platelets count in group 3 and 4 treated with carrot extract + olive oil and carotenoid complex + olive oil compared to the control group.

From the analysis, it can be concluded that carotenoid complex from GNLD as well as carrot extracted carotenoid, have haematomodulatory potentials in terms of increase in Hb (that makes them good in treatment of erythropoeitc proporphyria, which is a photosensitivity disorder), and the WBC count which is responsible for the enhancement of the immune system function, biological antioxidant function, by protecting cells and tissues from the damaging effect of free radicals and singlet oxygen as well as anticarcinogenic activity in certain circumstances .

From this work it is observed that carotenoid as a precursor of vitamin A plays an essential role in enhancing the immune system. It is advisable that fruits and vegetables which are rich sources of carotenoids be incorporated in the diet, to maintain overall good health and prevent chronic disease.

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