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Renoprotective effects of carotenoid on the Kidneys of Adult Wistar Rats

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Abstract- Caroteniods, the colorful plant pigments some of which the body can turn into Vitamin A, are powerful antioxidants that help prevent some forms of cancer, heart diseases and act to enhance immune response to infections. The aim of this study is to investigate the effects of oral administration of carotenoid on the kidney of adult wistar rats. Twenty healthy wistar rats weighing between 150-210Kg were used. They were divided into four groups (A, B, C & D) of five animals each. Group A served as the control and received 0.35ml of distilled water; the experimental groups B, C & D were orally treated with 0.4ml, 0.5ml and 0.6ml of carotenoid respectively for twenty one days. Twenty four hours after the last administration, the animals were weighed, anaesthetized using chloroform inhalation method and dissected. Kidney tissues were removed, weighed and trimmed down to a size of 3mm $\times$ 3mm and fixed in zenkers fluid for histological studies. The final body weight of the experimental groups increased significantly ($P< 0.001$) relative to the control (A). The relative kidney weights of the experimental groups are statistically similar with the control. Histological results proved that no histopathological lesions were observed in the experimental groups when compared with the control. The present study proved that consumption of carotenoid at low and high doses may not put the kidney at risks of adverse histopathological condition.

Index Terms- Carotenoid, kidney weight, body weight, Wistar rats, Hepatoprotective

I. INTRODUCTION

Carotenoids are a class of more than 600 natural occurring pigments synthesized by plant, algae, and photosynthetic bacteria.

These richly colored molecules are the sources of the yellow, orange and red colours of many plants [1].

Fruits and vegetable provide most of the carotenoids in human diet. Alpha carotene, beta-carotene, beta-cryptoxanthin, lutein, lycopene and zeaxanthin are the most common dietary carotenoids. Alpha carotene, Beta carotene and Beta cryptoxanthin are provitaminA carotenoids [2].

Carotenoids can be broadly classified into two classes, carotenes (alpha-carotene, beta-carotene, lycopene) and xanthophylis (beta-crytoxanthin, lutein and zeaxanthin) [3].

In pants, carotenoids have the important antioxidant function of quenching (deactivating) singlet oxygen, an oxidant formed during photosynthesis [4].

Test tube studies indicate that lycopene is one of the most effective quenchers of singlet oxygen among carotenoids [5]. Although important for plants, the relevance of singlet oxygen quenching to human health is less clear. Test tube studies indicated that carotenoids can also inhibit the oxidation of fats under certain conditions but their actions in humans appear to be more complex [6].

Carotenoids have many physiological functions. Given their structure, carotenoids are efficient free radical scavengers and they enhance the vertebrate immune system. There are several dozen carotenoid in foods people consume and most carotenoids have antioxidant activity [7].

Epidemiological studies have shown that people with high beta carotene intake and high plasma levels of Beta carotene have a significantly reduced risk of lung cancer. However, studies of supplementation with large doses of beta carotene in smokers have shown an increase in cancer risk possibly because Beta-carotene under intense oxidative stress e.g induced by heavy smoking gives breakdown products that reduce plasma VitaminA and worsen the lung cell proliferation induced by smoke [8].

More than 900 drugs, toxins and herbs have been reported to cause liver and kidney diseases. The kidney being the primary organ of drug and xenobiotics excretion is therefore liable to damage. This scenario provides a necessity to carry out research on the effects of carotenoids on the kidney of wistar rats.

II. MATERIALS AND METHODS

2.1: Breeding of Animals

Twenty wistar rats weighing between 150-210Kg were purchased from animal house of Anatomy Department, University of Calabar, Cross River State, Nigeria. They were allowed for seven days acclimatization under normal temperature ($27^{\circ}C$-$30^{\circ}C$), and fed ad libitum with water and guinea feed pallets from Agro feed mill Nigeria Ltd.

2.2: Drugs preparation

Commercial carotenoid was procured from Golden Neo-life Diamite (GNLD) Int. spartan by pharmaceutical contractors Isando road, Isando, South Africa and purchased from No. 6 Itu Road Uyo retail outlet, Akwa Ibom State, Nigeria. One capsule of carotenoid containing 900mg was dissolved in 10mls of distilled water and administered to the animals.

2.3: Experimental Protocols

The twenty adult wistar rats were weighed and assigned into four groups of five animals each. Group A as the control received 0.35ml of distilled water; the experimental groups B, C & D received 0.4ml, 0.5ml and 0.6ml of carotenoid respectively for a period of twenty one days. Twenty four hours after the last administration, the animals were weighed, anaesthetized under

the influence of chloroform vapour and dissected. Kidney tissues were removed, weighed trimmed down to a size of 3mm $\times$ 3mm thick and fixed in zenkers fluid for four hours for histological studies.

2.4: Tissue processing

The tissues passed through several processes of fixation, dehydration, clearing, infiltratrion, embedding, sectioning and staining. The fixed tissues in zenkers fluid were washed overnight under a sream tap water. Dehydration of the fixed tissues were carried out in different percentages of aclcohol 50%, 70% and 90% absolute. The dehydrated tissues were cleared in xylene for two hours after which infiltration was done in molten paraffin wax at a temperature of 60 $^{\circ}C$ for two hours each in two changes and then sectioned. Haemtoxylene and eosin method was used.

III. RESULTS

3.1 Morphometric Analysis of Body Weights

Table 1: Comparison of mean initial and final body weight and weight change in all the groups (A, B, C & D).

(Mean $\pm$SEM given for each measurement)

	GP A	GP B	GP C	GP D	F-RATIO	PROB OF SIG
INITIAL BODY INT	190.10$\pm$3.60	192.80$\pm$4.60	195.60$\pm$ 6.60	198.40$\pm$7.20	64.230	< 0.001
FINAL BODY INT	200.40$\pm$5.50	209.30$\pm$2.70	212.30$\pm$4.20	215.20$\pm$2.50	40.240	< 0.001
WEIGHT CHANGE	10.10$\pm$2.20	17.10$\pm$4.60	17.70$\pm$6.20	17.60$\pm$6.50	7.280	< 0.001

The final body weight for the experimental groups B, C, & D increased significantly ($P < 0.001$) relative to the control (A).

3.2: Morphometric analysis of kidney weight

Table 2: Comparison of mean relative kidney weight of all the groups (A, B, C & D)

(mean $\pm SEM$ given for each measurement)

	GP A	GP B	GP C	GP D	F. RATIO	PROB OF SIG.
KIDNEY WT	5.30$\pm$0.230	5.34$\pm$0.280	5.36$\pm$0.420	5.39$\pm$0.610	52.40	< 0.001

The relative kidney weights for the experimental group increased significantly ($p < 0.001$) with the control.

3.3: Histopathological Findings:

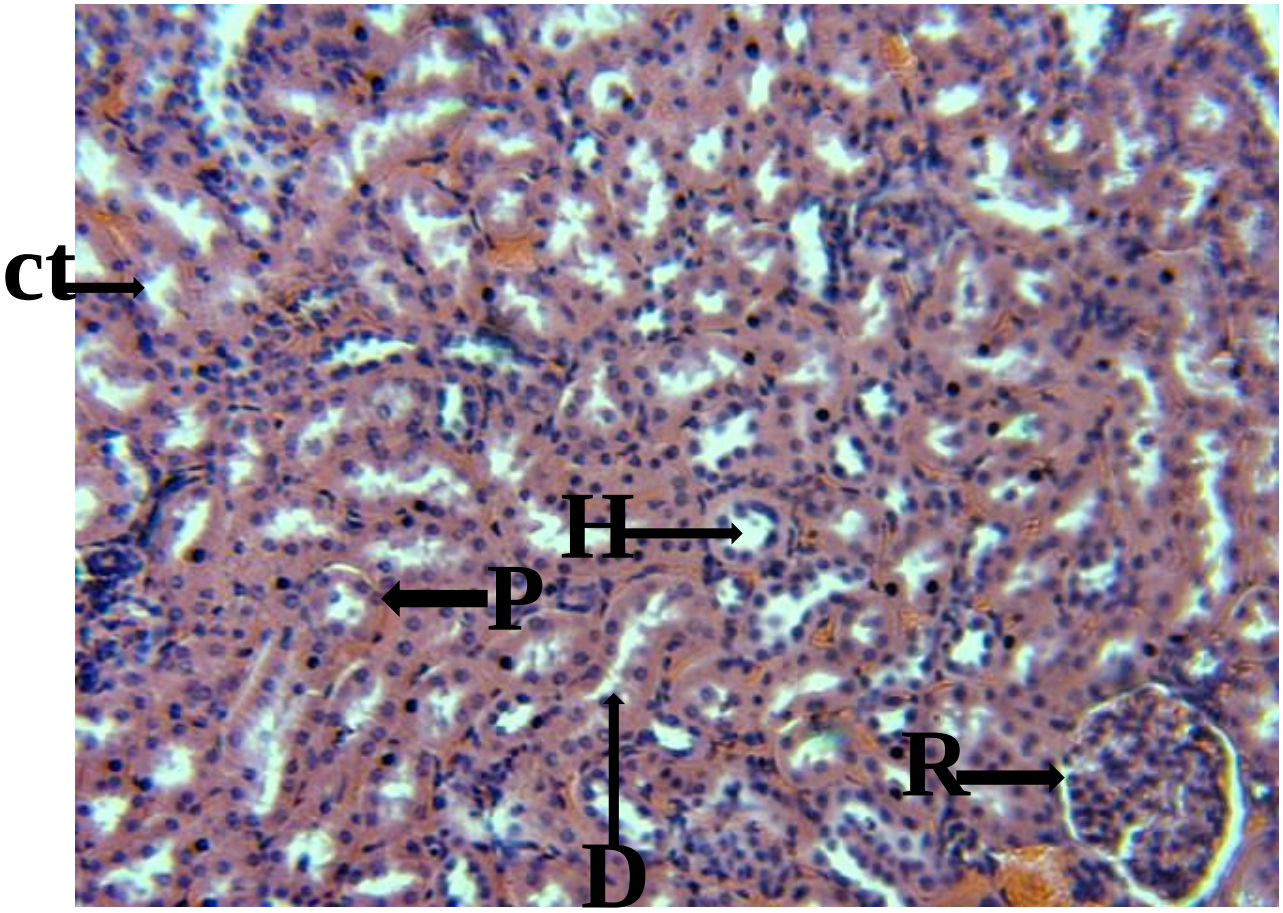

Fig 1, Micrograph 1 (control), shows normal histological structure of renal corpuscle (R), proximal convoluted tubule (P),distal convoluted tubule (D), henles loop (H), and collecting tubule (ct), stained by H & E technique, x 200.

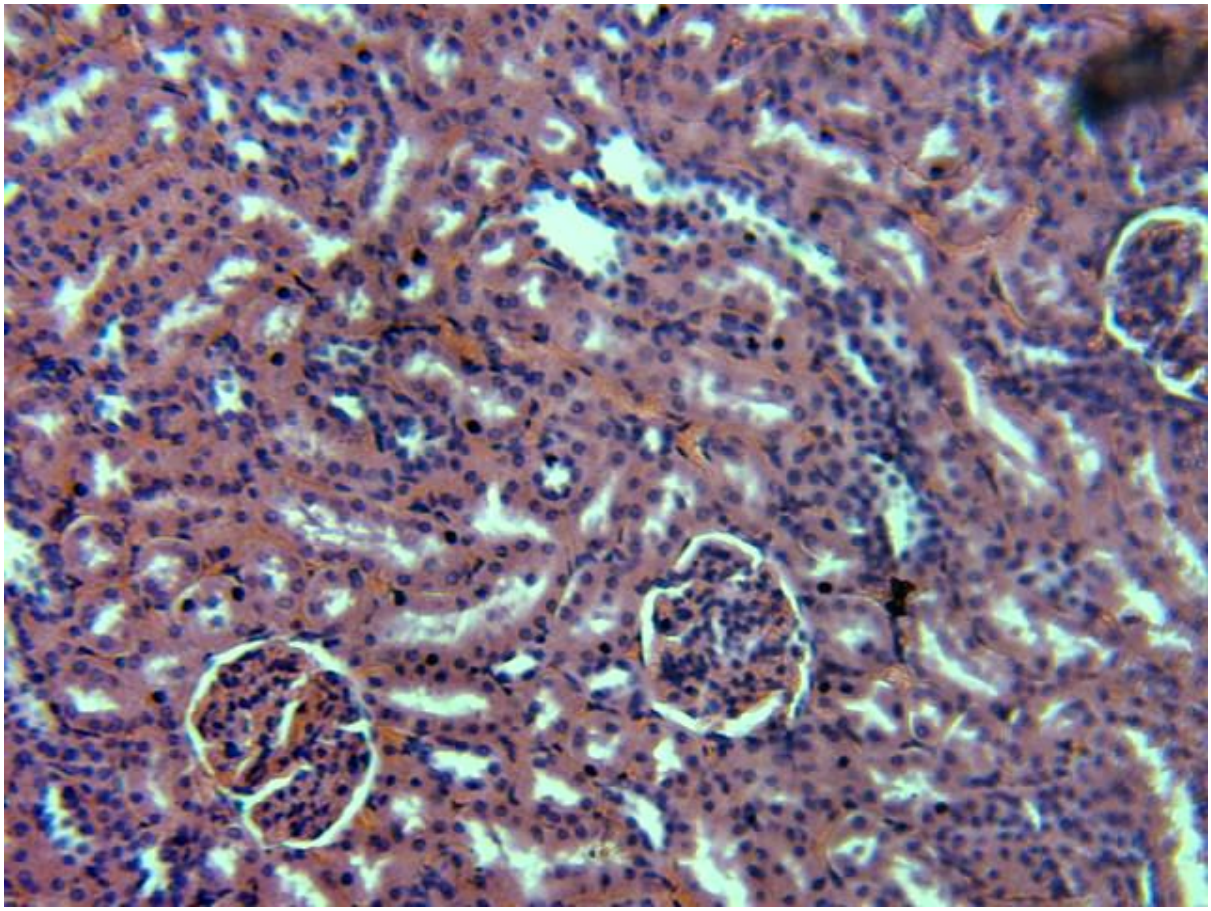

Fig 2, Micrograph 2 Group B, (treated with 0.4ml of Carotenoid) showing normal histoarchitecture of the kidney, stained by H & E technique, x 200.

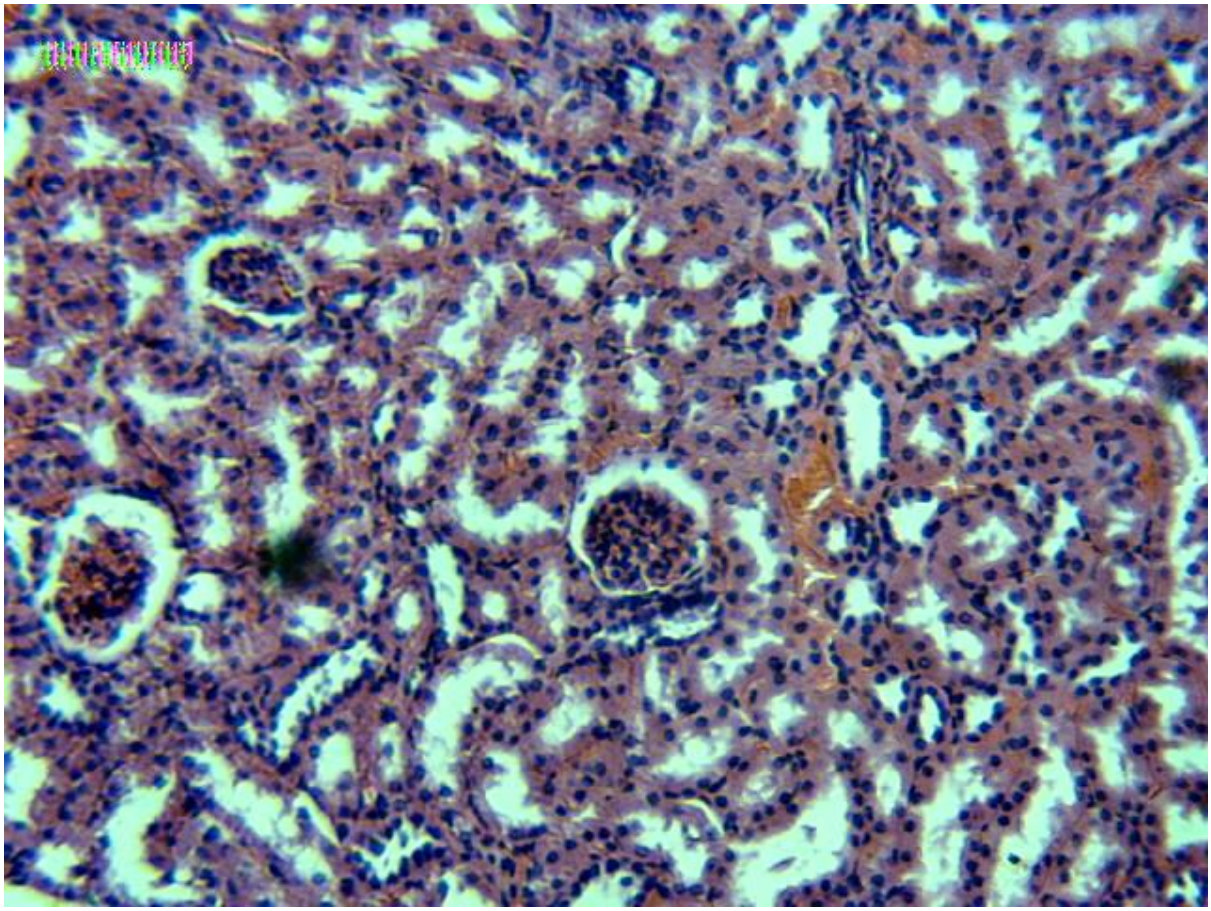

Fig 3, Micrograph 3 Group C, (treated with 0.5ml of Carotenoid)shows none distortion of the histoarchitecture of the kidney, stained by H & E technique, x 200.

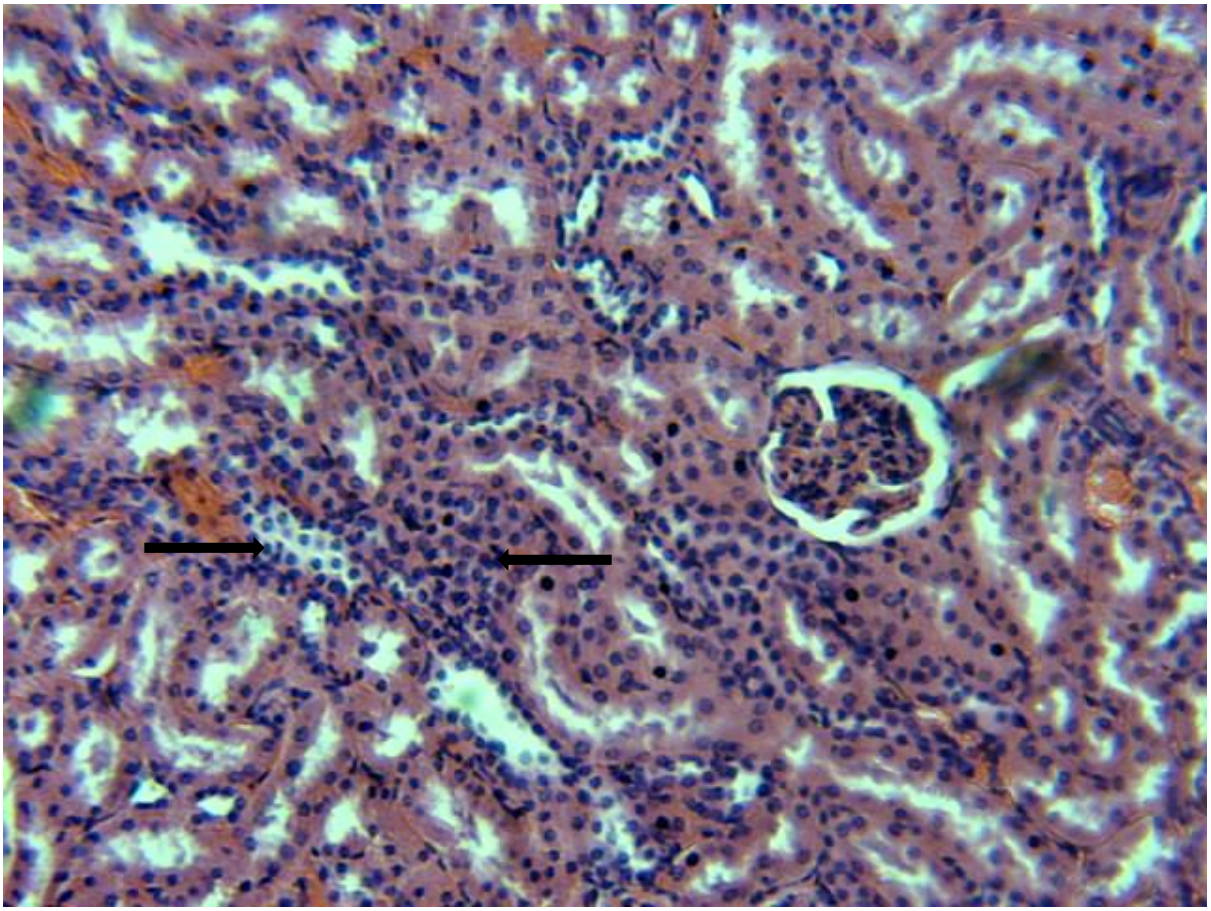

Fig 4, Micrograph 4 Group D, (treated with 0.6ml of Carotenoid),showing normal histological structure of the kidney, though, there is evidence of mild congestion and infiltration of cells into the tubules(Arrows), stained by H & E technique, x 200.

IV. DISCUSSION

In recent years there is an upsurge in the areas related to newer developments in prevention of disease especially the role of free radicals and antioxidants. Antioxidants are substances that neutralize free radicals or their actions. Nature has endowed each cell with adequate protective mechanisms against any harmful effects of free radicals: Superoxide dismutase, glutathione peroxidase, glutathione reductase, thioredoxin, thiols and disulfide bonding are a chian-breaking antioxidant which prevents the propagation of free radical reactions in all cell membranes in the human body. Ascorbic acid is also part of the normal protecting mechanism. Other non-enyzmatic antioxidants include carotenoids, flavonoids and related polyphenols, glutathione etc[9].

There is evidence to suggest that carotenoid act as modulators of intracellular of redox status.

There is ability to function as antioxidant has been known for many years. They are not just "another group of natural pigments", they are substances with very special and remarkable properties that no other group of substances possesses and that form the basics of their many varied functions and actions in all kinds of living organisms. The conjugated double bond structure is primarily responsible for the ability of beta carotenoid to quench singlet oxygen physically without degradation, and for the chemical reactivity of Beta carotene with free radicals such as the peroxyl, hydroxyl, and superoxide radicals.

Carotenoid have been shown to be able to prevent or decreases oxidation damage to DNA,lipid and proteins[10,11].

Oxidative stress and free radical attack on biological structure are believed to be the major factors in the initiation and propagation of the development of many degenerative diseases. In general, carotenoids behave as effective antioxidants in vitro[12, 13] and clear evidence exits from a majority of epidemiological studies on the incidence of CVD indicating an inverse relationship with dietary carotnoids[14] and circulating carotenoid levels. Carotenoid may function as chian breaking antioxidant reducing lipid peroxidation of such vulnerable membrane.

The antioxidant properties of carotenoids are primarily associated with their ability to quench singlet oxygen[15] and scavengers free radicals[16, 17].

Hence the results of the present study is in line with previous researches on antioxidant and hepatoprotective properties possessed by carotenoid.

V. Conclusion

From the present study, we therefore inferred that carotenoid has an appreciable ability to prevent damage to the kidney of humans.

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