JJBS

Jordan Journal of Biological Sciences

Volume 12, Number 3,August 2019
ISSN 1995-6673
Pages 283 - 288

Beta (β)-Carotene-Induced Effects on the Hepato-Biochemical Parameters in Wistar Rats Fed Dietary Fats

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Received August 4, 2018; Revised September 29, 2018; Accepted October 18, 2018

Abstract

Beta-carotene (βC), a lipid-lowering agent, has been proposed to be a lipid-soluble antioxidant that functions as a precursor of vitamin A. The aim of this study is to evaluate β-carotene- induced effects on hepato-biochemical parameters of Wistar rats exposed to dietary fats. Thirty Wistar rats were divided into six groups. Group A received rat chow, Group B received a high- fat diet, Group C received 300mg/kg body weight (b.wt) of βC, Group D received a high-fat diet for twelve weeks then 300mg/kg b.wt of βC for two weeks, Groups E received 300mg/kg b.wt of βC for two weeks followed by a high-fat diet for twelve weeks while Group F received a high-fat diet for twelve weeks followed by 150mg/kg b.wt of βC for two weeks. At the end, the animals were sacrificed; the blood and liver were collected for analyses while some tissues were fixed for histological studies. The results showed that the level of liver enzymes and bilirubin were increased in Group B compared with the Control (P<0.05). The histological examination showed the liver with fatty accumulation, infiltration by inflammatory cells and fatty vacuolation in the experimental Groups compared to the Control. The results suggest that the β carotene extract can be effective in treating the fatty liver disease.

Keywords: Liver, β-carotene, Dietary fat, Biochemical Parameters, Enzymes, Oxidative stress.

1. Introduction

Dietary fat and its effects on health and diseases have attracted interests for research. Fat is an important source of energy, and facilitates the absorption of fat-soluble dietary components such as vitamins (Javier and Carmen, 2012). An uncontrolled intake of dietary fats could lead to obesity, type 2 diabetes mellitus, dyslipoproteinaemia, hypertension, and metabolic syndrome including the coronary heart disease, strokes and cancer (Wolfram *et al*., 2015). Lipids and insulin play important roles in regulating blood sugar, and altered levels of fat deposits can release triglycerides and free fatty acids into the blood, causing hyperlipidemia (Madubunyi *et al*., 2012). Hyperlipidemia and oxidative stress are major risk factors for atherosclerosis, and are the most important risk factors for cardiovascular diseases (Madubunyi *et al*., 2012; Li *et al*., 2013). Cardiovascular diseases constitute one of the largest public health problems in the world today and are responsible for more than seventeen million deaths annually (WHO, 2005). Moreover, chronic disorders such as cardiovascular diseases, type 2 diabetes mellitus, hyperlipidemia, and obesity are the risk factors for non-alcoholic fatty liver disease (de Alwis and Day, 2009). Non-alcoholic fatty liver disease (NAFLD), regarded as the hepatic manifestation of the metabolic syndrome, currently represents the most common cause of chronic liver diseases (Chalasani *et al*., 2012). NAFLD ranges from simple hepatic fat accumulation (steatosis) to non-alcoholic steatohepatitis (NASH), where fat is accompanied by hepatocyte injury and necroinflammation which pose as increased risk factors for liver cirrhosis and hepatocellular carcinoma (Chalasani *et al*., 2012). Depending on the assessment tools, the prevalence of NAFLD in adults ranges between 20 % and 30 %, reaching up to 46 % in some studies (Bellentani *et al*., 2010). Approximately, 70 % of patients living with NASH also have concurrent dyslipidemia; hence, making treatment with a lipid-lowering medication appear to be a reasonable approach (Williams, *et al.*, 2011; Hyogo *et al*., 2008).

Studies have shown that high-fat diets can easily induce obesity (French and Robinson, 2003), while epidemiological studies have shown that when the average amount of fat in the diet increases, the incidence of obesity also increases (Saris *et al*., 2000). This has led to a worldwide effort to decrease the amount of fat in the human diet. Diets rich in fat not only induce obesity in humans, but also make animals obese (Buettner *et al*., 2007). There is a positive relationship between the level of fat in the diet and body weight in both rats and mice

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(Ghibaudi *et al*., 2002). High-fat diets with fats at 50 % of the total energy in weanling mice, have been used to induce obesity that was called nutritional obesity; the model was later renamed dietary obesity (Fenton & Dowling, 1953). It has been reported that despite the growing problem of obesity, Canadians and Americans are eating less fat than a generation ago (Lissner *et al*., 2000). This shows that the increasing rate of obesity cannot be totally explained by high intakes of fat in the diet, suggesting that the type of fat may also play a role (Moussavi *et al.,* 2008). Some studies have reported that not all fats are obesogenic, and the dietary fatty acid profile, rather than the amount of energy from fat, is an important variable in developing dietary obesity (Ellis *et al*., 2002; Kien *et al*., 2005).

Other factors that may contribute to obesity induced by a diet rich in fat include failure to adjust the oxidation of fat to the extra fat in the diet, increase in adipose tissue lipoprotein lipase activity, increased meal size and decreased meal frequency, overconsumption of energy attributed to high energy density of the diet, orosensory characteristics of fats and poorly satiating properties of the high-fat diets (Buettner *et al*., 2007; Kiess *et al*., 2008). Adipose tissues are considered endocrine organs that secrete cytokines; thus obesity could possibly be regarded as a chronic inflammatory disease (Kiess *et al*., 2008). In the rats fed diets that are high in fat, a linear increase in body fat with an increasing body weight has appeared. The results of the study of Woods et al. (2003) showed that measuring body fat is a more sensitive method for assessing obesity in animals. Tulipano *et al*. (2004) categorized rats that are fed high-fat diets based on their final body weight, with rats in the highest quartile, designated as obesity-prone, and those in the lowest quartile designated as obesity-resistant.

For many decades, medicinal plants have been used to prevent or treat various diseases (Tapiero *et al*., 2004) and some have been used throughout the world for their hypoglycemic, hypolipidemic, or antioxidant activities (Tapiero *et al.,* 2004). Recent studies have shown that carrots have higher concentrations of beta carotene than other dietary sources (Bayerl, 2008). The aim of this study was to evaluate the β-carotene-induced effects on hepato biochemical parameters in the liver of Wistar rats exposed to dietary fats.

2. Materials and Methods

2.1. Preparation of the Extract

Fresh carrots were purchased from Meat Market Abakaliki, Ebonyi State, Nigeria. The carrots were dried under shade for three weeks and were grounded into powder. The pulverized carrots were wrapped in Whatman filter paper and placed in the chamber of Soxhlet extractor. Then 250ml of N-Hexane was added into the Soxhlet flask and placed on a heating mantle. The solvent was heated at 50^0C, the Soxhlet extractor condenses the sample in the filter paper, and the content of the carrots were extracted until a clear solvent started coming out of the extraction chamber. The extract was concentrated using a water bath at 50^0C, and was then stored in the refrigerator.

2.2. Animal Procurement

Ethical approval was arranged and obtained from the Federal University Ndufu Alike Ikwo Ethics and Animal Handling Committee. Thirty Male Wistar rats with average weight of 71.05g were procured from and maintained in the animal house of the Department of Biological Sciences, Federal University Ndufu-Alike Ikwo, Ebonyi State Nigeria. The animals were housed in metal cages, and were given access *ad libitum* to food and water with acclimatization period of two weeks.

2.3. High-Fat Diet Preparation

Cow fat was purchased from Meat Market Abakaliki, Ebonyi State, Nigeria. The fat was dissolved by heating, collected in metal containers and stored in the refrigerator. A high-fat diet was prepared by mixing 60 % of the cow fat and 40 % of normal rat chow as described by Ghibaudi *et al.* (2002) and was then stored in the refrigerator.

2.4. Animal Experimentation

The rats were randomly divided into six groups of five rats in each. Group A (Control) received normal rat chow for fourteen weeks. Group B received a high-fat diet (HFD) daily for fourteen weeks. Group C received 300mg/kg body weight (b.wt) of β-Carotene daily for fourteen weeks. Group D received a high-fat diet daily for twelve weeks followed by 300mg/kg b.wt of β-Carotene daily for two weeks. Group E received 300mg/kg b.wt of β-Carotene daily for two weeks and then HFD daily for twelve weeks. Group F received HFD daily for twelve weeks followed by 150mg/kg b.wt of β-Carotene daily for two weeks. After fourteen weeks of administration, the animals were weighed and humanely sacrificed by cervical dislocation. Blood was collected through cardiac puncture. The animals were dissected, and the liver was harvested, weighed and fixed in 10 % formal saline for a histological examination. The liver weight index in percent (%) was calculated as liver weight over body weight multiplied by 100, as described by Sayed *et al.* (2015). The liver tissues were processed and embedded in paraffin wax. Thin sections of 5 μm thick were made, stained using haematoxylin and eosin (H&E) and examined under a light microscope.

2.5. Biochemical Study

The biochemical parameters studied included serum alanine aminotransferase (ALT), aspartate amino-transferase (AST), Gama Glutamyl Transferase (GGT), alkaline phosphatase (ALP). The activity of ALT was determined using Reitman-Frankel colorimetric method according to the methods of Reitaman and Franke (1957) as modified by Sayed *et al.* (2015) using a Quimica Clinica Applicada (QCA) test kit. ALT activity was measured by monitoring the concentration of pyruvate-hydrazone formed with 2, 4-dinitrophenylhydrazine which is proportional to the concentration at 505nm. The serum was separated by centrifugation at 3600 rpm for fifteen minutes for the determination of serum Gamma glutamyltransferase (GGT) levels using Quimica Clinica Applicada (QCA) commercial test kits according to the Manufacturer's instruction. AST activity was determined by the Reitman-Frankel colorimetric method using a Quimica Clinica Applicada (QCA) test kit. AST activity was measured by monitoring the concentration of

oxaloacetate hydrazone formed with 2, 4–dinitrophenyl hydrazine spectrophotometrically at 505nm. Alkaline phosphatase acts upon the AMP-buffered sodium thymol phthalein monophosphate. Addition of the alkaline reagent stops the enzyme activity and simultaneously develops a blue chromagen which can be measured photometrically at the wavelength of 550nm. Bilirubin content was assayed and estimated according to the Manufacturer's instruction, and the absorbance of the sample against the blank was read at 560nm and calculated.

2.6. Data Analysis

All data were expressed as mean ± SD. The level of homogeneity among the groups was tested using one way Analysis of Variance (ANOVA). Where heterogeneity occurred, the groups were separated using Duncan Multiple Range Test. A value of $P < 0.05$ was considered to indicate a significant difference between groups. Data analysis was done using Statistical Package for Social Sciences (version 20.0).

3. Results

3.1. Liver Weight Index

The results showed a significant increase in the liver weight index of the animals in Group B when compared with those of the Control group (Group A) (P<0.05). There was a significant decrease in the liver index of the animals in Group D when compared with the animals in Group B, but there was a significant increase in the liver index of the animals in Group D when compared with those animals in the Control Group (Group A) (P<0.05). However, there was a significant decrease in the liver index of the animals in Group C when compared with the animals in Group A and other groups (P<0.05) as shown in Figure 1.

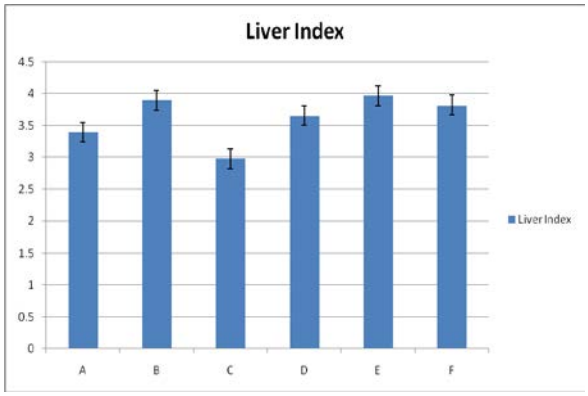

Figure 1. Showing the Liver Index of the animals in different groups

3.2. Effects on Hepatic Function

The serum levels of ALT, ALP, GGT, and Bilirubin were significantly increased (P<0.05) in Group B animals when compared with the animals in the Control (Group A), but there was a significant decrease in the serum level of AST in the animals in Group B when compared with the animals in the Control (Group A) (P<0.05). The results showed a significant decrease in the serum levels of ALP, ALT, AST, GGT, and Bilirubin in the animals in Groups D and F when compared with the animals in Group B (P<0.05) as shown in Table 1.The results also showed that there was no significant difference in the parameters for animals in Group D when compared with the animals in Group E as well. Also, there was no significant difference in the parameters for the animals in group A when compared with the animals in Group C (Table 1).

Table 1: Effect of HFD and Beta Carotene on the hepatic function

GP	ALT (U/L)	AST (U/L)	ALP (U/L)	GGT (U/L)	Total Bilirubin (mg/dl)
A	31.68 ± 0.74*	100.66 ± 0.77	72.56 ± 0.77	11.71 ± 0.84	0.22 ± 0.02*
B	44.68 ± 0.60	83.80 ± 2.39*	79.82 ± 0.98	43.21 ± 2.69**	0.57 ± 0.05**
C	29.72 ± 2.00*	105.86 ± 2.19	64.17± 0.05*	11.62 ± 0.86	0.23 ± 0.00*
D	40.17 ± 0.08	69.67 ± 0.65*	76.67 ± 5.00	22.86 ± 4.73*	0.34 ± 0.02
E	40.72 ± 0.69	68.17 ± 1.50*	74.87 ± 5.14	26.31 ± 1.69*	0.35 ± 0.02
F	42.72 ± 0.72	86.26 ± 8.55	78.17 ± 0.05	41.62 ± 0.72**	0.44 ± 0.02**

Values are expressed as Mean ± SD; N=5, *P<0.05. **

3.3. Effects on Oxidative Stress and Lipid Peroxidation

The results of the present study showed that hepatic superoxide dismutase (SOD) and catalase (CAT) activities in the animals of Group B were significantly decreased when compared with the animals in the Control Group (P<0.05). Malondiadehyde (MDA) levels of the animals in Group B were significantly increased in animals when compared to the animals in the Control Group (P<0.05) (Table 2).

However, the results showed that the oxidative stress and lipid peroxidation markers in the hepatic tissues were restored with a significant increase in SOD and CAT activities, whereas the MDA levels were significantly decreased with the administration of β-Carotene to the animals in Group D when compared with the animals in Group A (P<0.05) as shown in Table 2. The results also showed no significant difference in the SOD and CAT activities and the levels of MDA in the animals in Group C when compared to the animals in Group A as shown in Table 2.

Table 2. Effect of HFD and Beta Carotene on lipid peroxidation and oxidative stress markers

Group	MDA (nmol/mg pro)	SOD (U/mg pro)	CAT (U/mg pro)
A	0.56 ± 0.06**	28.18 ± 2.91**	18.06 ± 0.07**
B	3.77 ± 0.75*	14.00 ± 1.98*	7.17 ± 0.08*
C	0.32 ± 0.12	32.78 ± 3.73	19.81 ± 0.55**
D	1.81 ± 0.42	19.83±0.95*	15.76 ± 2.05**
E	1.76 ± 0.77	20.76 ± 0.63	15.81 ± 0.56**
F	3.12 ± 0.01	18.73 ± 1.99*	11.31 ± 1.26

Values are expressed as Mean ± SD; N=5, *P<0.05 **

3.4. Histological Evaluation

The results showed liver sections from the animals in Group A (Control) with normal hepatic cells radiating from the central vein with well-preserved cytoplasm and nucleus as shown in Figure 2A. The histology of the liver

of animals in Group B showed hepatic tissues with steatosis due to the presence of fat accumulations resulting in hepatocytes vacuolation as in Figure 2B. Meanwhile, the liver section of the animals in Group C showed normal hepatic architecture (Figure 2C). The results showed that the degree of hepatic injury including steatosis, hepatocytes with cytoplasmic vacuolation and lobular inflammation were to a lesser degree in the animals in Groups D and E as shown in Figures 2D and 2E. Thus steatosis with inflammation and hepatocytes with cytoplasmic vacuolation were attenuated with beta carotene for two weeks. The results also showed that beta carotene (Group F) had been shown to reduce the level of the distortions to the hepatic tissues as shown in Figure 2F.

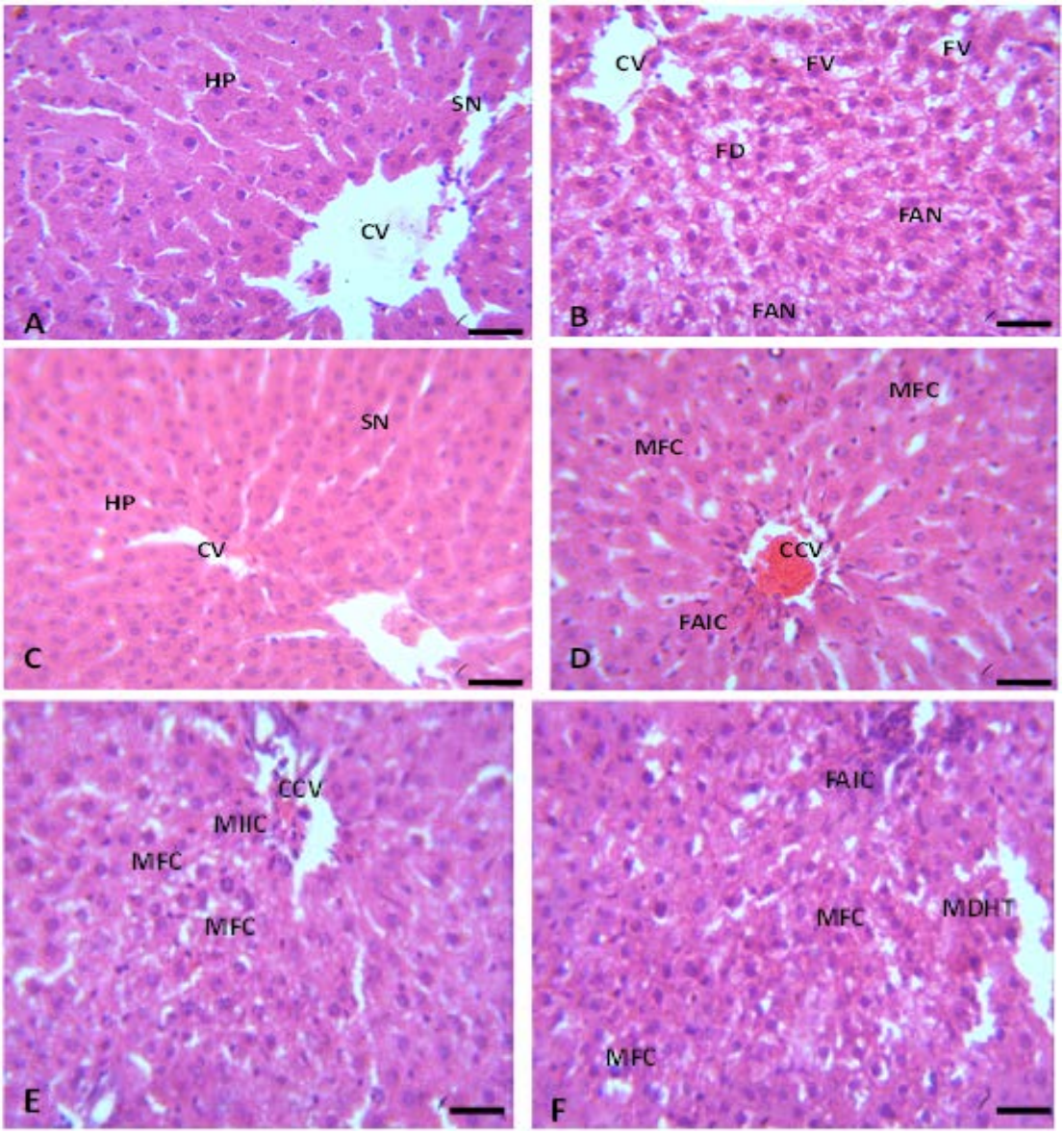

Figure 2. (A) Section of the Liver of Group A (Control) animals showing normal hepatic architecture with central vein (CV) sinusoids (SN) and hepatocytes (H); (B) Section of the Liver of Group B animals showing fatty changes (FC), fatty necrosis, and distortion of hepatic tissue; (C) A section of the liver of Group C showing normal hepatic architecture with central vein (CV), hepatocytes (H), and Sinusoids (S); (D): Section of the liver of Group D showing mild fatty changes (MFC), focal aggregate of inflammatory cells (FAIC), and congestion of central vein (CCV); (E) A Section of the liver of Group E showing mild fatty changes (MFC) and moderate infiltration of inflammatory cells (MIIC); (F) Sections of the liver of Group F showing, moderate regeneration with mild fatty change (MFC), focal aggregate of inflammatory cells (FAIC), and mild distortion of hepatic tissue (MDHT). Stain: H & E; Magnification: x400. Scale Bar: 1mm=5µm

4. Discussion

The results of this study showed an increase in the liver weight index in animals fed with dietary fats alone for twelve weeks, while the liver weight index was significantly reduced with the administration of beta-carotene for two weeks. This showed that β-Carotene can be active in reducing the fat deposits in the liver, and thereby decreasing the hepatic index.

The present study has shown that β-Carotene administration resulted in a significant decrease in liver enzymes when compared with the animals fed with dietary fats. This is in agreement with the study of Vardi *et al.* (2010) who reported that β-Carotene given for twenty-one days before the methotrexate application provided significant protection from hepatotoxicity resulting from

methotrexate intoxication. Chung *et al*. (2009) had reported that in hyperlipidemic patients, there was a clinically significant elevation in the levels of AST/ALT, and this was in agreement with the results from the present study. It has been shown that visceral fats release free fatty acids which are transported to the liver by the portal, vein and may contribute to hepatic steatosis, the production of triglyceride rich in very low density lipoprotein (VLDL). The elevated β-oxidation can contribute to the significant elevation of the liver AST/ALT (Chung *et al*., 2009).

However, the elevated serum liver enzymes namely AST, ALT, ALP, GGT, and Bilirubin levels, were reversed upon treatment with beta-carotene, and this was in accordance with Jensen (2008), who observed that most of the carotenoids appeared to be inversely correlated to the fat mass, suggesting that in obesity, carotenoids are sequestered in adipose tissues thereby decreasing their plasma concentrations. Moreover, van Helden *et al*. (2011) had demonstrated that the anti-obesity effects of β-carotene were linked to its pro-vitamin A effects which means that β-carotene exerts its effects by functioning as a precursor of vitamin A. Thus β-carotene functions as a lipid-lowering agent and a lipid-soluble antioxidant. The results also showed that the administration of beta-carotene before feeding the rats with dietary fats helped to protect the liver against damage by maintaining the levels of the serum liver enzymes, lipid profile, and adipocytokine markers (Okechukwu *et al.*, 2018).

The observed elevation in all the liver enzymes in animals fed with dietary fats was also in agreement with AL-Dosari *et al*. (2011), who had reported that there were increased plasma activities of AST, ALT, ALP and GGT in the fat diet-fed rats. ALT and AST are biomarkers in the diagnosis of hepatic damage because they are released into the circulation after hepatocellular damage (Naik and Panda, 2007). ALT and AST are liberated into the blood whenever liver cells are damaged, and thus resulting in increased plasma enzyme levels, and has been shown to be a very sensitive index of liver damage (Edwards *et al*., 2008; Crook, 2012). Although none of these enzymes are specific to the liver, but ALT occurs in much higher concentration in the liver than elsewhere. Therefore, the increased serum ALT level in the present study, more specifically, reflects hepatic cellular damage (Edwards *et al*., 2008; Crook, 2012; Ekandem and Peter, 2015).

The results obtained from the present study revealed that intake of dietary fats at 60 % fat levels for twelve weeks have been shown to have damaging effects on the liver of Wistar rats with the distortion of the liver cellular architecture, dilation of the hepatocytes with congestion of the central vein, dilation of the sinusoidal spaces, and infiltration of the inflammatory cells with cytoplasmic ground glass appearance of the hepatic tissues. The results showed that the hepatic injury was ameliorated by the administration of beta-carotene for two weeks. This was in agreement with the report given by Sayed *et al*. (2015). Meanwhile, the result also revealed that there have been dose-dependent effects of beta-carotene on the liver, as the administration of 300mg/kg body weight of beta-carotene showed a better treatment outcome to the hepatic injury caused by the fat diets than the 150mg/kg body weight of beta-carotene. However, the pre-treatment of beat-carotene to the Wistar rats before exposure to dietary fats has a protective effect against hepatic damage. The major drawback of the present research is the small sample size of five animals used in a group which stand as a major and important limitation to the study. Therefore, further larger scale studies are needed to verify and solidify the findings.

5. Conclusion

The consumption of β Carotene-rich foods, fruits and vegetables have been shown to have enhancement effects in modulating the hepatic functions and histological structures related to liver damages especially when it relates to the nonalcoholic fatty liver disease. This may be due to their synergistic anti-oxidative, anti-inflammatory, and lipid-lowering effects in the body while the pre-treatment of beta-carotene before a dietary fat intake protected the liver from the manifestation of the hepatic injuries in the Wistar rats, βeta carotene could be recommended as a remedy for the treatment of the nonalcoholic fatty liver disease, especially that resulting from the high-fat diet consumption.

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