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IMPACT OF ROOIBOS TEA ON EXPERIMENTAL MODEL OF HIGH-FAT DIET-INDUCED NON-ALCOHOLIC FATTY LIVER DISEASE

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Abstract

Rooibos tea obtained from the leaves of *Aspalathus linearis,* is a popular caffeine-free beverage with abundant flavonoids. Previous studies have reported antioxidant, anti-inflammatory and hepatoprotective effects. However, its effect on NAFLD rat model has not been explored. The present study therefore investigated the therapeutic effects of rooibos tea on high-fat diet (HFD)-induced NAFLD in rats. Twelve male albino rats were divided into three equal groups (A, B and C) (n=4) namely: Control, HFD and HFD + Rooibos tea (RBT) respectively. High-fat diet was prepared daily by mixing 40% Lard with 60% standard rat diet. Group A rats received a standard rat chow and water whereas groups B and C were given HFD daily for 28 days. Rats in Group C received RBT *ad libitum* from Day 15 to 28. On the last day of treatment, relative liver weights (RLW), serum biochemical liver markers and liver histomorphology were assessed. Results revealed that HFD feeding significantly increased serum alanine transaminase (ALT) and alkaline phosphatase (ALP) levels. Administration of RBT significantly decreased ALT, ALP and RLW, but increased total bilirubin levels. Aspartate transaminase and conjugated bilirubin were not altered significantly in different groups. Microscopical examination of the liver of HFD group showed severe hepatic degeneration, necrosis and inflammatory cellular infiltration. However, liver histology of HFD+RBT group showed significantly decreased lesions as compared to HFD group. In conclusion, the outcomes from the present study indicate that rooibos tea is efficient in inhibiting adverse hepatic damage caused by high-fat diet in rats.

Keywords: *Rooibos tea; Liver; Histopathology; Serum biochemistry; High-Fat Diet; NAFLD*

Introduction

Rooibos (*Aspalathus linearis*) is an indigenous South African herbal tea and its commercial production started more than a century ago (1). Several biological activities of rooibos including anti-mutagenic, anti-carcinogenic, anti-allergic, chemopreventive, antioxidant, antidiabetic, hypoglycemic, anti-obesity, cardio-protective, antihypertensive, anti-inflammatory, antispasmodic, bronchodilatory, antiviral, antimicrobial effects have been reported (2,3). The hepatoprotective effect of rooibos tea on animal model of hepatic injury using a chemical hepatotoxicant has also been reported (4) but there is paucity of scientific literature on its effect in non-alcoholic fatty liver disease.

Nonalcoholic fatty liver disease [NAFLD] is a major public health concern and has become one of the most prevailing causes of chronic hepatic diseases in children and adults (5). The exact mechanism of NAFLD is unknown but it involves a spectrum of hepatic injury associated with fat deposition, in the absence of alcohol consumption, which progresses from steatosis to non-alcoholic steatohepatitis, fibrosis and finally to hepatic cirrhosis (6). The goals for treatment of NAFLD are basically preventive or by reversing liver injury and fibrosis (7) but these have achieved little success and no specific therapy can be recommended to NAFLD patients (8). Researches geared towards developing new potential therapeutic targets for the treatment of NAFLD have remained crucial in order to identify promising approaches.

Therefore, based on the foregoing, the present study aimed at investigating a potent agent for the treatment of NAFLD from a commonly consumed herbal tea, rooibos (RBT). Evaluation of the effect of sub-acute administration of RBT on liver histology and liver function biochemical markers of rats with HFD-induced NAFLD was hereby conducted.

Methods

Rooibos Tea Preparation

Commercial [Freshpak®] rooibos tea by Entyce Beverages (Rivonia, South Africa) was purchased from a retail supermarket within Enugu metropolis, Enugu State, Nigeria. The aqueous extract of the tea was prepared daily by boiling 2g of the dry tea in 1500ml of distilled water for 10min. The mixture was allowed to stand subsequently for 20 min to cool down to room temperature. The mixture was then filtered to separate the insoluble residue and the filtrate obtained was used for the experiment.

High-Fat-Diet Preparation

The High-Fat Diet (HFD) was prepared by mixing 40% fat (lard-saturated fatty acid) with 60% commercial standard chow diet (Top Feeds® limited, Ibadan, Nigeria).

Animals

Twelve (12) albino rats weighing between 100 - 120g were used for the study. The rats were obtained from the Animal House of Department of Physiology, University of Nigeria Enugu Campus (UNEC) and were housed at the Animal House of Anatomy Department, UNEC in well ventilated steel cages to acclimatize for one (1) week. Animals were kept under controlled standard environmental conditions (12hr:12hr dark-light cycle, room temperature of 23±1°C and 55±3% humidity) and fed with standard rat chow and tap water *ad libitum.* All animal experiments were approved by our Institutional Ethics Committee and were conducted in accordance with the International guidelines and principles for the care and use of laboratory animals in research.

Experimental protocol

The animals were randomly divided into three (3) groups [A - C] of four (4) rats each. After acclimatization, rats in groups B and C were placed on high-fat-diet (HFD) *ad libitum* for 28 days. Rats in Group A (control) were fed only standard rat chow and water *ad libitum*. On Day 15, rooibos tea (RBT) was provided for rats in Group C as drinking water and this treatment lasted for 14 days.

Biochemical analysis

At the end of the treatment (Day 28), after an 8-hour fast, blood samples were obtained through retro-orbital puncture of the median canthus of the eye into plain dry bottles. The samples were kept undisturbed for 30minutes for clotting and thereafter centrifuged for 15 minutes at 3000rpm to separate the serum for biochemical assay. Sera obtained were used for the estimation of serum

alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), total (TB) and conjugated bilirubin (CB) levels using standard methods.

Histological studies

Liver tissues were excised from the rats after sacrifice under mild chloroform anaesthesia. The liver of each rat was weighed, prior to fixation, to determine the change in organ weight with respect to body weight at time of sacrifice (relative liver weights). The tissues were subsequently fixed in 10% formal saline prior to further histological processing using the paraffin embedding technique for light microscopy. Sections of 3-5 microns were obtained using a rotary microtome. The sections were stained with Haematoxylin and Eosin (H and E) staining technique as described by Baker *et al.* (9). The liver sections were examined using an Olympus™ binocular microscope with in-built lighting system and photomicrographs were captured with AmScope microscope eyepiece camera.

Statistical analysis

Data were expressed as the mean value ± S.E.M and were analyzed using the SPSS Inc. software version 20 (SPSS Inc, Chicago, Illinois). Hypothesis testing was conducted using one-way analysis of variance [ANOVA] followed by Tukey's post hoc test and students' *t* test for multiple comparison. Probability values of less than 0.05 was considered to indicate statistical significance.

Results

Effect of Rooibos tea on serum biochemical levels and Relative liver weight

In HFD rats (Group B), there was increase in levels of all serum biochemical parameters assayed but statistically significant levels were observed only in ALT and ALP ($p<0.05$) when compared with control (Table 1). ALT and ALP of HFD+RBT rats (Group C) were significantly reduced to values similar to control ($p<0.05$). However, TB levels were significantly increased upon RBT treatment when compared with both control and HFD groups ($p<0.05$).

Relative liver weight was not altered significantly upon HFD feeding. However, significant reduction was observed in rats treated with RBT when compared with both control and HFD groups ($p<0.05$).

Histological findings

Figure 1 shows the centrilobular regions (A) and periportal regions (B) of the liver sections from rats in control (normal diet), HFD and HFD+RBT groups (Groups A, B and C respectively). Normal histoarchitecture of the hepatic tissue was observed in rats fed with normal diet. Feeding of rats with HFD produced extensive tissue degeneration. However, upon treatment with RBT, there was evidence of good tissue preservation from hepatic damage induced by HFD.

Discussion

Animal models of HFD-induced NAFLD have been used to assess the potency of hepatoprotective agents. In the present study, a high-fat diet (HFD) was used to induce hepatic damage in albino rats and the hepatoprotective activity of Rooibos tea (*Aspalanthus linearis* leaves) against the liver injury was investigated. As observed, a HFD containing 40% fat content exerted histoarchitectural changes consistent with non-alcoholic fatty liver disease. Here, our data demonstrated that RBT treatment prevents hepatocyte ballooning degeneration, inflammation and, generally, liver injury in the HFD-induced NAFLD model.

The degenerative changes of the liver parenchyma observed in the liver tissues of HFD-only fed rats occurred as a result of hepatic fat (lipid) accumulation, which is pivotal to liver injury. Serum biochemical findings also correlated with the histopathology of the liver and these findings connote impaired hepatocytes' integrity as a result of HFD feeding. Consumption of a High-fat diet is well known to affect liver metabolism leading to development of fatty liver (steatosis) by a complex mechanism. The oxidative capacity of hepatocyte mitochondria becomes impaired due to the lipid accumulation and this consequently leads to increased generation of reactive oxygen species causing oxidative stress and cell death (10).

Rooibos treatment, in this study, was observed to ameliorate the lesions exerted by HFD on the liver of

treated rats. Marked preservation of the hepatic tissue, typified by little or no inflammatory cellular infiltrates, necrosis and ballooning degeneration, was observed. More so, serum biochemical parameters showed evidence of protection in concordance with the histological findings as the serum levels of liver enzymes (ALT, AST and ALP) were restored to values near normal. This protective effect of RBT against NAFLD may probably have been effected via antioxidant and/or anti-inflammatory mechanism. Previous studies have demonstrated that the flavonoids in *Aspalathus linearis* (Aspalathin and nothofagin) have shown antioxidant and anti-inflammatory effects (11, 12). It is possible that the various compounds in RBT may have either acted singly or interacted in combination resulting in the hepatoprotective effect observed in this study. Free radicals scavenging activities of herbal teas have been attributed to their polyphenolic components (13,14), and their involvement in the management of many oxidative stress-related diseases have been documented (15).

Conversely, the raised level of total bilirubin, observed with HFD feeding, were not restored by RBT treatment, rather, further increased level was observed. A possible explanation to this effect cannot be proffered. Moreover, no previous data has documented raised levels of serum bilirubin after rooibos tea treatment. It is not clear, however, what type of bilirubin (conjugated or unconjugated) is associated with NAFLD (16), however, the conjugated bilirubin estimated in this study remained unaltered either with HFD feeding or RBT treatment. Interestingly, previous studies have reported that serum bilirubin significantly contributes to total antioxidant capacity (17,18) and have also shown cytoprotective effects (19). It may be assumed that there is a possibility that the protective effects of RBT as observed in the present study may have also been contributed by the raised TB levels. Further investigations to identify this matter are therefore pertinent to discover the underlying effect and mechanism. Nonetheless, since the liver histomorphology of treated rats were preserved by RBT treatment in this study, it is a strong indication that the extract ameliorates hepatic injury induced by the high-fat diet. Similar hepatoprotection have been previously documented upon treatment with rooibos following hepatic injury induced by hepatotoxicants (4, 20).

In conclusion, the present study reveals that Rooibos tea (*Aspalathus linearis*) possesses hepatoprotective effects and may be used to treat hepatic injuries induced by high-fat diets. The preservation of the hepatocytes by the Rooibos tea may have been through a mechanism that either disrupts the generation of reactive oxygen species or the cellular changes induced by HFD. Undoubtedly, rooibos tea enhanced the structural integrity of hepatocytic cellular membrane in order to preserve it from damage caused by the fat diet. Further studies to elucidate the mechanism(s) of hepatoprotective effect of *A. linearis* in HFD-induced non-alcoholic fatty liver will be required in future.

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Table 1: Effects of Rooibos tea on serum biochemical parameters and relative liver weight of rats with HFD-induced NAFLD

Groups	Parameters					
	Aspartate Transaminase (iu/l)	Alanine transaminases (iu/l)	Alkaline phosphatase (iu/l)	Total Bilirubin (mg/dl)	Conjugated Bilirubin (mg/dl)	Relative Liver Weight
ND	170.15±3.79	74.85±6.91$^{\#}$	480.45±19.42$^{\#}$	0.44± 0.08	0.20± 0.05	4.64±0.22
HFD	208.25±30.86	98.60±7.73*	1511.03±26.45*	0.62± 0.05	0.26± 0.07	3.94±0.25
HFD+RBT	159.48±10.02	74.63±2.48$^{\#}$	463.08± 39.23$^{\#}$	0.98±0.13*$^{\#}$	0.42± 0.11	2.68±0.36*$^{\#}$
F-ratio	1.848	5.012	412.972	8.760	1.900	11.295
Sig.	0.213	0.034	0.000	0.008	0.205	0.002

Data expressed as Mean±SEM. * and $^{\#}$ = p<0.05 when compared to ND and HFD controls respectively.
[ND: Normal diet; HFD: High-Fat Diet; RBT: Rooibos Tea]

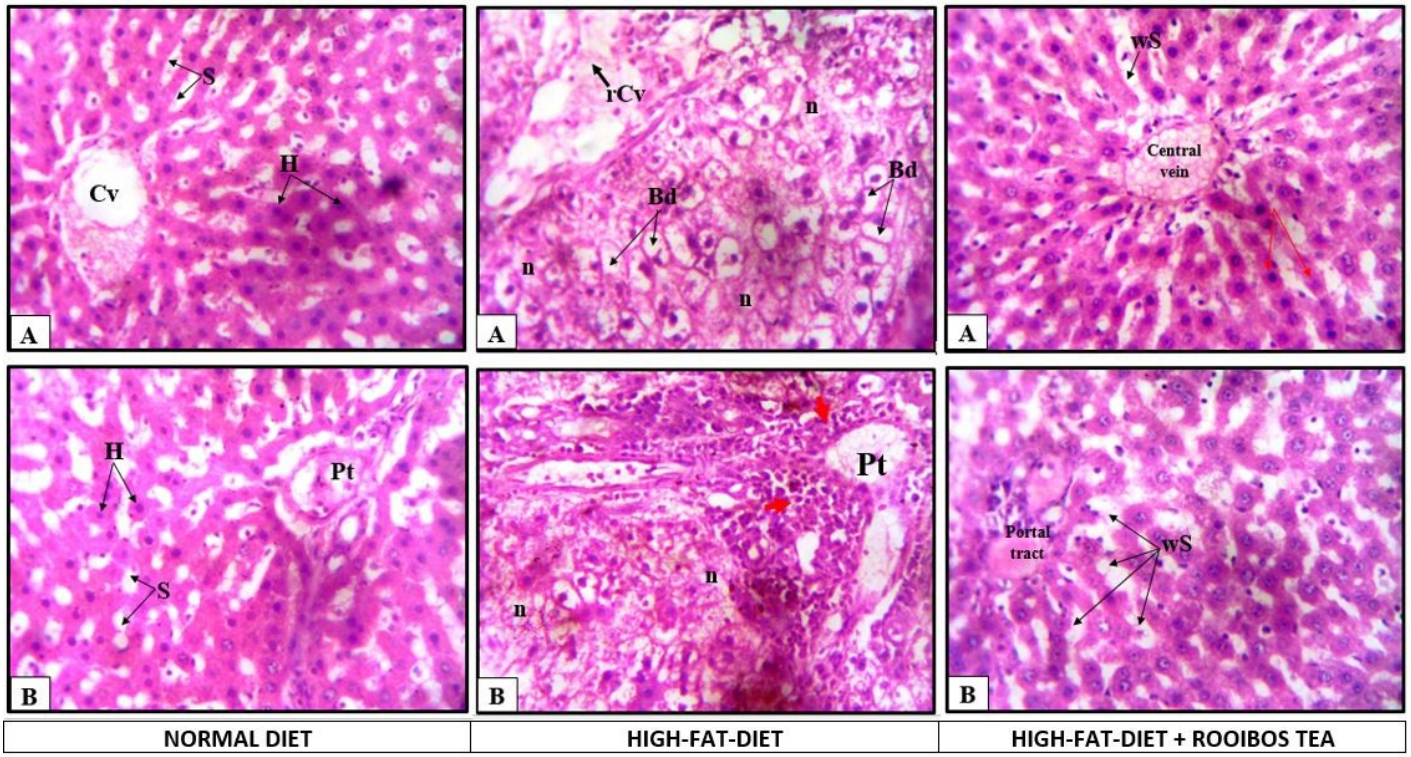

Figure 1: **Light photomicrographs of histological sections from excised liver tissues showing centrilobular (A) and periportal (B) regions of the hepatic lobules.** *Normal Diet*: Liver section photomicrograph of normal control rat showing normal central vein (Cv), portal tract (Pt), and sinusoidal spaces (S) flanked by plates of hepatocytes (H). *High-Fat-Diet***:** Liver sections showing ruptured central vein (rCv), enlarged portal tract (Pt) with infiltration of inflammatory cells (red arrows), hepatocytes necrosis (n) and ballooning degeneration (Bd). *High-Fat-Diet+Rooibos tea:* Liver sections showing evidence of good tissue preservation from hepatic damage. Mild shrinkage of hepatocytes is noted (red arrows) with resultant widening of the sinusoidal spaces (wS). [Stain: H&E/Mag: A&Bx400]