Acta Medica et Biologica
Vol. 44, No. 3, 147-151, 1996

Hepatoprotective Effects of Buckwheat Extract in Rabbits Fed on a High-fat Diet

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Received February 20 1996; accepted May 23 1996

Summary. **The effect of buckwheat extract (BE) on the content of total cholesterol, triglyceride and ascorbate free radicals in liver homogenate, and on macroscopic and microscopic pictures of the liver in animals receiving a high-fat diet (HFD) was observed. Male mongrel rabbits were randomly divided into three groups: 1) control, 2) animals given HFD containing cholesterol and coconut oil, 3) rabbits treated with HFD + BE over a period of 12 weeks. Contents of total cholesterol and triglyceride in the liver of animals on BE decreased. The number of ascorbate free radicals, examined *in vitro*, in the liver of these animals was markedly elevated. Protective effect of BE against changes induced in the liver of animals receiving a HFD was confirmed by macroscopic and microscopic examination of the organ.**

Key words—buckwheat, hepatoprotection.

INTRODUCTION

Chronic liver disease may result from a wide variety of infectious, chemical, or autoimmune insults. Recent investigations have increased our understanding of the pathological mechanisms responsible for hepatocellular injury in several areas, but there have been few significant therapeutic advances.[1)] Steatosis is defined as an accumulation of triglycerides in the cytoplasm of cells in which little or none is found in the normal state.[2)] Hepatic cells are considered to be steatotic when the fat accumulation becomes visible on examination by light microscopy.

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Buckwheat leaves (Fagopyrum esculentum M. and Fagopyrum tataricum G) are a potentical source of the industrial extraction of rutin.[3)] Buckwheat leaves are likely to affect lipid metabolism disturbances.[4)]

The objective of the present study was to determine the effect of buckwheat extract on liver damage induced by a high-fat diet in rabbits.

MATERIALS AND METHODS

Three groups of ten male mixed breed rabbits with a mean body weight of 3.65 kg (range 1.9-4.9) fed a standard basic laboratory LSH diet (Factory of Chow, Motycz) were used. The animals were randomly assigned to the following groups: Group 1, controls; Group 2, high-fat diet (HFD); Group 3, HFD and buckwheat (Herba Fagopyri esculenti) extract (Fagorutin-Fink Naturarznei GmbH, Herrenberg, Germany) 0.25 g/kg/24 h, given orally. The HFD consisted of (g/kg body weight/24 h): cholesterol (0.5), hydrogenated coconut oil (0.5), cholic acid (0.1). Buckwheat extract (BE) was mixed with the basic diet and given every morning as a pellet to the fasted rabbits. The experiment lasted 12 weeks. Animals were weighed every 2 weeks. At the end they were deprived of food for 18 h and then randomly sacrified by exsanguination.

Blood samples were taken for biochemical assays by heart puncture and the liver was removed. Basic laboratory biochemical tests of the blood serum (total protein, creatinine, urea, glucose, aminotransferases) were performed using an RA 1000 laboratory automation (Technicon, U.S.A.).

Electron spin resanance (ESR) spectra, corresponding to ascorbate free radicals, were recorded in the liver samples *in vitro* at ambient temperatures, using

a Radipan SE/X 2544 spectrometer (9.4 Ghz, 3 mW, 100 kHz amplitude mondulation of 0.25 mT, scan 50 mT, time constant 1 s, time scan 4 min) and 1, 1-diphenyl-2-picrylhydrazyd as standard. Liver samples were dried in a vacuum and ground.[5]

Liver homogenates were prepared in 0.9% saline to assay total cholesterol and triglycerides, extracted with chloroform/ethanol (2 : 1, v/v). Test kits from Boehringer, Mannheim, were used. The liver was weighed and evaluated macroscopically and microscopically. Sepcimens for histopathological studies were always taken from the same place in the liver. Fresh, unifixed pieces of the liver were freeze-sectioned and stained with Oil Red O for the presence of neutral lipids. Other pieces were fixed with Carnoy solution, with sections were stained with hematoxylin and eosin, and PAS method for glycogen content.

Statistical analysis of the results was performed by means of an IBM computer using the Student's t-test for unpaired samples. Results were expressed as means ± standard error (SE).

RESULTS AND DISCUSSION

The body weight of rabbits increased to where, after 12 weeks, it was 13% higher than initially. No significant differences in weight gain were found among the groups.

Basic laboratory tests (total protein, creatinine, urea, glucose, aminotransferases) did not reveal any essential abnormalities as compared with the control group.

Table 1 demonstrates the concentration of spins calculated per gram of dried liver tissue. The process of free ascorbate radical formation, examined *in vitro*, was reduced in rabbits exposed to a HFD by 21%, compared with control animals. However, the number of these radicals was markedly increased in rabbits maintained on the high-fat diet and simultaneously receiving BE. The concentration of spins in animals of Group 3 was even higher than in the control rabbits.

In the liver homogenate of animals receiving HFD, the concentration of investigated lipid fractions was significantly increased: total cholesterol by 341% and triglyceride by 78% (Table 2). The content of lipids in the liver homogenate of rabbits given a HFD and BE was depressed, but the change did not reach the control level. Triglyceride content was diminished significantly.

A macroscopic picture of the liver showed profound changes in rabbits fed a HFD. The livers of the HFD administered animals appeared yellowish and slightly paler than those of the remaining animals: the capsule of the liver was taut, with yellow points appearing through it; in cross-section the organ was yellowish and rough. The appearance of the liver taken from the rabbits given BE was almost normal.

Microscopy study revealed the following pictures in particular groups of animals:

Table 1. Ascorbate free radicals (ASC) in liver (*in vitro*)

Group		ASC (10^{16} spins/g dry tissue)
1. (Control)		1.24±0.28
2. (High-fat diet-HFD)		0.98±0.21
3. (HFD+buckwheat extract)		1.47±0.28
p	1/2	>0.4
	2/3	>0.1

Values are means ± SE from 10 animals.
P-statistical comparisons among groups.

Group 1

Hepatocytes of rabbits in the control group showed normal morphological structure (Fig. 1). They contained glycogen grains in the zone of permanent

Table 2. Content of total cholesterol (Ch) and triglyceride (TG) in the liver and liver weight index (LWI) with statistical comparison among the groups

Group		Ch (mg/g)	Tg (mg/g)	LWI (g/kg body weight)
1. (Control)		3.51±0.36	10.63±0.84	24.67±1.04
2. (High-fat diet-HFD)		15.42±2.78	18.87±1.88	28.55±1.61
3. (HFD+buckwheat extract)		11.72±1.63	14.53±1.42	28.15±1.14
p	1/2	<0.01	<0.01	>0.05
	2/3	>0.2	<0.05	>0.05

Values are means ± SE from 10 animals.
P-statistical comparisons among groups.

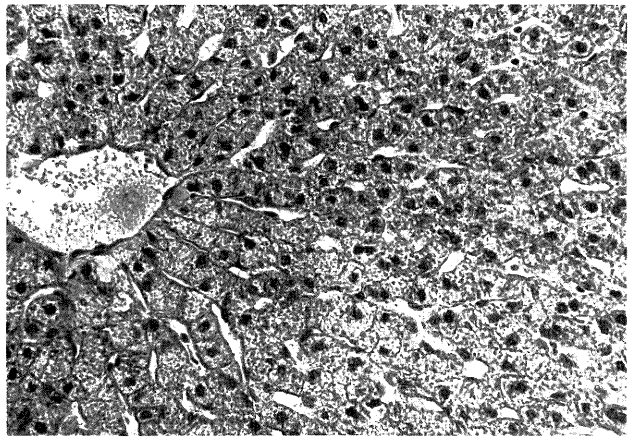

Fig. 1. Normal hepatic lobule of rabbits in the control group. Hematoxylin-eosin, ×280

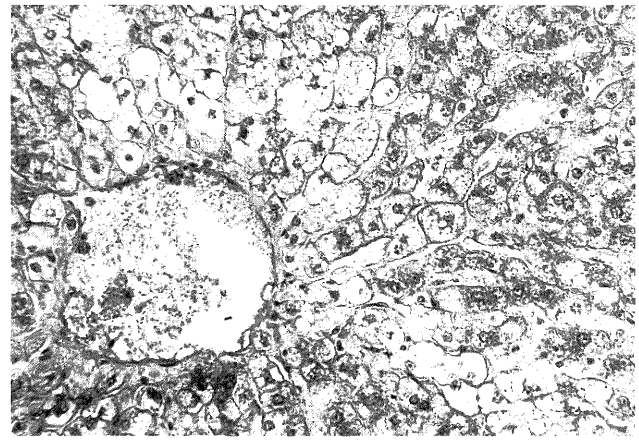

Fig. 2. Emptiness of the cytoplasm and foamy cytoplasm in the hepatocytes of rabbits fed a HFD; in the permanent repose zone and in varying activity one. Hematoxylin-eosin, ×280

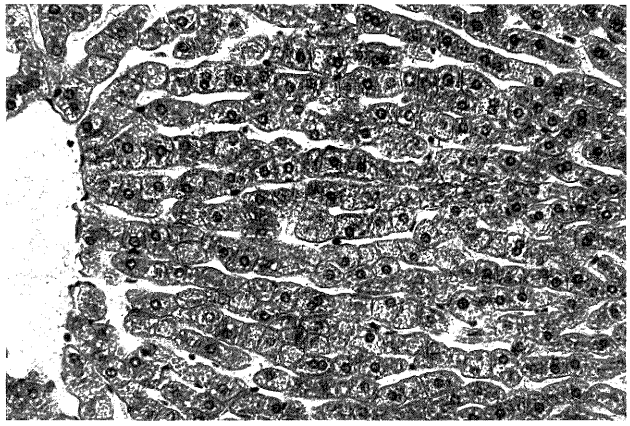

Fig. 3. Fine, foamy cytoplasm in the hepatocytes of rabbits fed a HFD with buckwheat extract added; in the permanent repose zone, as well as in the zone of varying activity. Hematoxylin-eosin, ×280

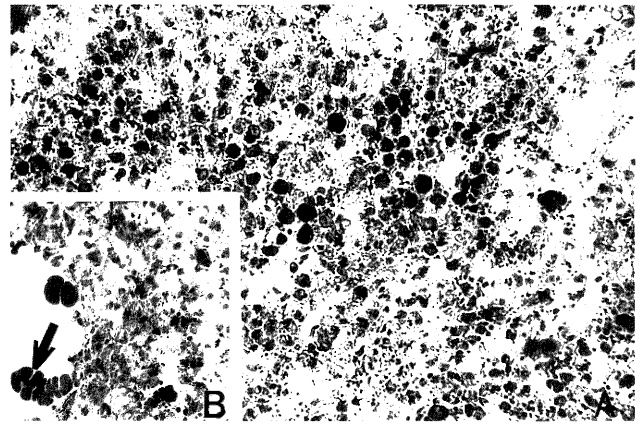

Fig. 4. Large amounts of lipids in the form of big colliquative drops in the cytoplasm of hepatocytes and in the lumen of the vessels of rabbits fed a HFD, frequently in the entire lobule (**A** ×120) and in the lumen of the vessels (**B**) *(arrow)*. Oil red O, ×250

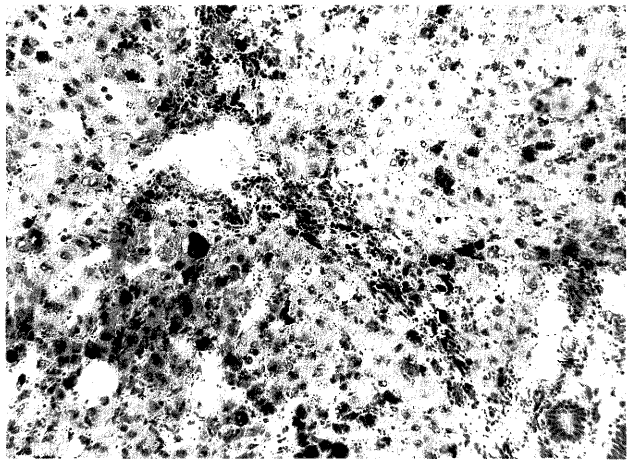

Fig. 5. Accumulation of minute lipid droplets in hepatocytes of rabbit fed a HFD and simultaneously receiving buckwheat extract. Oil red O, ×120

repose and in the varying activity zone in the hepatic lobules. Lipids were present in the Disse spaces in the form of very tiny droplets, and sometimes in the cytoplasm of the hepatocytes.

Group 2

In rabbits fed a high-fat diet (HFD), the hepatocytes disclosed both emptiness and a foamy cytoplasm in the permanent repose zone and in varying activity one (Fig. 2). Lipids were found in extremely large amounts (Fig. 4) in the form of fine but mainly large, colliquative drops in the cytoplasm of the hepatocytes, most frequently in the entire lobule. Lipid drops appeared in Disse's spaces, Kupffer cells, and in the lumen of the vessels. Hepatocytes of these rabbits had more glycogen than hepatocytes of control group animals, and they accumulated in the zone of permanent repose as well as in the zone of varying activity.

Group 3

Hepatocytes of rabbits being fed a HFD with Fagopyrum extract added displayed, in the majority of cases, morphological changes which were markedly less as compared with the hepatocytes of rabbits fed a HFD (Fig. 3). Hepatocytes exhibited fine, foamy cytoplasm and the accumulation of minute lipid droplets in the permanent repose zone (Fig. 5). In some hepatocytes, there was cytoplasmic emptiness and large drops of lipids in the cells of the permanent repose zone. Glycogen was seen to appear in hepatocytes of the rabbits in this group in very low amounts, mainly around the central vein.

Our previous investigations revealed that the rabbits maintained on the high-fat diet and simultaneously receiving the buckwheat extract demonstrated slightly reduced concentrations of malondialdehyde in their blood plasma.[6] In this study there were morphological changes in the liver. Extremely large amounts of lipid appeared in the hepatocytes, Kupffer cells and Disseis spaces as well. Also, glycogen was present in large amounts in the cytoplasm of hepatocytes. There was a smaller amount of lipids and glycogen in hepatocytes of rabbits fed a HFD with the buckwheat extract.

In the present experiment we demonstrated a content of free ascorbate radical in the liver of rabbits *in vitro* that corresponds to the condition *in vivo* with respect to the concentration of ascorbic acid. Ascorbic acid is considered to be the body's major defence mechanism for protection against free OH and O_2 radicals.[7] Free radicals are molecules or parts of molecules which tend to be particulary reactive because they possess an unpaired electron the outer orbital.[8] Among the chemical antioxidants, vitamin C is regarded as one of the most important in the prevention of lipid peroxidation.[9] Ascorbic acid acts as a strong reducing agent capable of reducing oxygen, nitrogen and sulphur-central radicals.[10] In addition to scavenging radicals directly, ascorbic acid is a key reductant which acts in concert with other chain-breaking antioxidants such as vitamin E and β-carotene.

The challenge of managing subjects with chronic liver disease is often quite complex. For the most part, one has only been able to provide symptomatic relief, but has been unable to halt the eventual progression to cirrhosis of many of the disoders.

There seem to be four possible explantations for the accumulation of fat in the liver: increased synthesis and decreased oxidation of fat by the hepatic parenchymal cells, increased mobilization of depot fat with accumulation in the liver, and the decreased release of fat from the liver cells to the general circulation.[11] Buckwheat leaves are a potential source for the industrial extraction of rutin. They are also likely to affect lipid metabolism disturbances.[12]

In summary, it should be emphasized that the administration of the buckwheat extract to rabbits kept on a high-fat diet antagonizes the diet-induced abnormalities in the liver.

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