

Biological studies of some nutritional formula on liver functions

Hanan, S.H.

Home Economic Dept. Fac. of Specific Education . Monifiya University

Nehad,R. EL-Tahan

Nutrition and Food Science Dep., Home Economics Faculty, Mounfia University, Egypt.

Abstract

The present study investigated the effects of some nutritional formula on liver functions in rats. Twenty one male albino rats were divided into (3) groups (7) rats in each group. The first group was control which fed on basal diet only as a negative control. The other groups were received basal diet containing 15% from formula (1) which contained 60 % Whey protein , 20 % Carrots and 20% Rhubarb and formula (2) contained 60 % Soy Protein , 20 % Hibiscus and 20% Turmeric. Liver functions was assessed by estimation of plasma concentration of enzymes activities of aspartate amino transferase (AST), alanine amino transferase (ALT), lipid fraction (total cholesterol and triglyceride) and cholesterol fraction (HDL-c, LDL-c, VLDL-c). Results showed an improvement in case of tested formula (2) followed by formula (1) for the above parameters. So, this study concluded that liver functions in rats can be ameliorated by administration of 15% from formula (2) which contained 60 % Soy Protein , 20 % Hibiscus and 20% Turmeric.

Key words: Nutritional formula, liver functions - cholesterol fractions.

Introduction

Phytotherapy is the treatment and prevention of diseases using plants or plants part, such as leaves, flowers, roots, fruits, seeds, and rhizomes. Preparation made from them called medicinal plants, or herbs (Weiss and Fintelmann, 2000). Many plants were suggested to ameliorate or care the liver diseases, among them were the birch, celandine, Dates palm, dates, rosemary, papaya, onion, Turmeric and lettuce (Morsi, 1992). Medicinal plants have very important place as they not only maintain the health and vitality of human beings and animals, but also cure several disease, including liver disorders without causing any toxicity (Govind and Madhuri, 2010).

Turmeric is one of the oxidation of human low-density lipoprotein (LDL) (Gulfranz et al., 2011).

The Rhubarb root contain a high, nicotinic acid (niacin) and vitamin A. The dietary fiber of 14 varieties of Rhubarb root has been shown to be as high as 6.4-11.5% depending on variety and degree of ripeness (Münzbergová, 2012).

Carrot (*Daucuscarota L.*) contained a several insults (Dias, 2012). The effect of aqueous suspension of Carrot (*Daucuscarota L.*) on carbon tetrachloride induced liver damage, CCL4 induced toxicity induced liver damage antagonize aqueous dose of 250-500 mg/kg suspension of Carrot (*Daucuscarota L.*) by raising the level of LDH (Lactate dehydrogenase) and lowering of Carrot (*Daucuscarota L.*) by raising the level of AST (aspartic transaminases) and ALT (L-alanine amino transfers) 5% seed of Carrot (*Daucuscarota L.*) given to albino mice to evaluate hepato protective action against dimethyl lami-noaze-benzen induced liver carcinogenesis was studied and the results showed significant changes in the plasma level of alanine (AST) alkaline phosphate (ALP), total protein and serum albumin which analyzed by malondialdehyde but there is no harmful effect of Carrot (*Daucuscarota L.*) on the liver moreover, it exerts hepatoprotective effect against hepatobiliary carcinogens because of their antioxidant property (Sun et al., 2009). So, the present study was carried out to investigate biological effects of some nutritional formula contained Carrot (*Daucuscarota L.*), Turmeric purpurea, Rhubarb root with soy protein and whey protein on serum parameters of liver functions in rats.

Material and Methods

Materials

Plants

The tested plants were obtained from local market in Shebin El-kom, Menofia Governorate, Egypt. Whey protein and soy Protein were obtained from National Research Center in Cairo.

Chemical reagents

Reagent kits were purchased from Diamond Diagnostics (Egypt).

Experimental animals

Twenty one white male albino rats weighing about $180 \pm 5g$ were used as experimental animals in the present investigation. They were obtained from the animal house of Research Institute of Ophthalmology, El-Giza, Egypt. They were kept under observation for one week (as adapted period) before the onset of the experiment. The animals were housed in stainless steel cages at normal atmospheric temperature ($25 \pm 5^\circ C$) and had a 12 h light-dark cycle. Food and water were consumed *ad libitum*.

Methods:

Preparation of plant powder

These plants were washed and dried in drying oven at $50^\circ C$ for 3 days, then crushed and milled as a dried powder.

Animals diet

The basal diet was prepared according to **AIN (1993)**. The vitamin mixture was prepared according to **Campbell (1963)**, while salt mixture was prepared according to **Hegsted *et al.* (1941)**.

Experimental design

Twenty one male albino rats ($180 \pm 5g$) were randomly divided into 3 equal groups (seven rats each). All rats were fed on basal diet for one week before starting the experiment for acclimatization. After the adapted period, the initial weight was $205 \pm 5g$. Groups of rats were as the follows:

Group (1): Rats ($n=7$) were fed on basal diet only as control negative group.

Group (2) :Rats ($n=7$) were fed on formula 1 which contained 60 % Whey protein , 20 % Carrots and 20% Rhubarb .

Group (3): Rats ($n=7$) were fed on formula 2 which contained 60 % Soy Protein , 20 % Hibiscus and 20% Turmeric.

By the end of the experimental periods (28 days),rats were scarified using diethyl ether anesthesia at fasting state . Part of the blood was taken to determine the level of serum glucose and other portion of blood samples was collected and allowed to coagulate at room temperature;other portion of blood was added to EDTA (ethylenediaminetetracetic acid) and centrifuged at 3000r.p.m for 15 minutes. Serum was carefully aspirated and transferred into clean covettubes and stored frozen at $-20^\circ C$ until the time of analysis.

Biochemical analysis:

Serum Alkaline phosphatase (ALP) was determined according to the procedure of **(IFCC methods., 1983)**. Aspartate amino transferase (AST) or (GOT) glutamic -oxalo acetic trans aminase and glutamic pyruvic transaminase (GPT) or Alanine amino transferase (ALT) were carried out according to the method of **Henry(1974) and Yound (1975)**. Glucose was determined by enzymatic test according to **Tietz (1976) and Yound (1975)**. Enzymatic colorimetric determination of triglycerides was carried out according to **Fassati and Prencipe (1982)**. Total Cholesterol was determined by colorimetric method according to **Allain (1974)**. The determination of HDL was carried out according to

the method of **Fnedewaid (1972)** and **Gordon and Amer (1977)**. The determination of VLDL (very low density lipoproteins) and LDL (low density lipoproteins) was carried out according to the method of **Lee and Nieman (1996)**. Total immunoglobulin (IgG, IgM, IgE and IgA) determined by Radioimmunoassay as described by the method of **Patrono and Peskar (1987)**.

Histopathological study: Livers of the scarified rats were dissected, removed, washed with normal saline and put in 10% formalin solution. The fixed specimens were then trimmed, washed and dehydrated in ascending grades of alcohol. The tissue specimens were cleared in xylene, embedded in paraffin, sectioned at 4-6 microns thickness, stained with Hematoxylen and Eosin (H and E) and then studied under an electronic microscope according to (**Carleton, 1979**).

Statistical analysis

Statistical analysis were done using the Statistical Package for the Social Sciences (SPSS for WINDOWS, version 11.0; SPSS Inc, Chicago). Comparative analyses were conducted using the general linear models procedure (SPSS Inc). Values of $P < 0.05$ were considered statistically significant.

RESULTS

1-Effect of feeding 15% tested formula on serum lipids in normal rats.

Administration of the tested formula at 15% level caused significant decreases in serum of total cholesterol, triglycerides, LDL-c and VLDL-c compared to control group (Table 1). Serum HDL-c levels increased but not significantly by the administration of the formula 1 (G2). Rats that were given formula 2 (G3) showed significantly higher levels of HDL-c compared to control group (G1). The value of other lipid parameters of formula 2 were higher than control group. The obtained results in the same line of **Crouse (1999)** who found that soy protein can decrease LDL-c, total cholesterol and increase the level of HDL-c. Also, **Gulfraz et al. (2011)** reported that tumeric reduce the absorption of lipids from diet.

Table (1): Effect of feeding 15% tested formula on serum lipids in normal rats.

Serum lipids	G1	G2	G3
Total cholesterol	75.43 ^a ±2.19	76.33 ^a ±3.15	75.47 ^a ±1.13
Triglycerides	76.48 ^b ±0.13	76.8 ^b 1.03±	81.4 ^a ±3.01
HDL-cholesterol	53.94 ^a ±0.12	47.87 ^b ±1.15	48.89 ^b ±0.04
LDL-cholesterol	20.2 ^c ±1.17	24.9 ^a ±4.34	22.5 ^b ±0.74
VLDL-cholesterol	1.29 ^b ±1.17	1.56 ^a ±4.34	1.28 ^b ±0.74

Values are mean±SD. Values in the same column sharing the same superscript letters are not statistically significantly different at ($p < 0.05$)

2--Effect offeeding 15% tested formula on serum lipids on liver functions in normal rats.

From data presented in table (2) the administration of formula 2 (G3) significantly reduced AST and didn't effected on ALT level when compared with the other treatment groups. There is no significant differences between group 2 with 3. From the above results, it could be noticed that crude fiber in legumes is a group of indigestible carbohydrates. It can improve the function of the alimentary tract and also lower blood glucose ,cholesterol levels and liver functions (**Roberfroid , 2000**).

Table (2): -Effect offeeding 15% tested formula on liver functions in normal rats.

Parameters	G1	G2	G3
AST(U/L)	27.8 ^b ±0.07	30.2 ^a ±1.11	32.5 ^a ±0.21
ALT(U/L)	19.8 ^b ±1.91	28.9 ^a ±1.41	27.4 ^a ±0.5

Values are mean±SD. Values in the same column sharing the same superscript letters are not statistically significantly different at (p<0.05)

3--Effect of feeding 15% tested formula on immunological productions in normal rats .

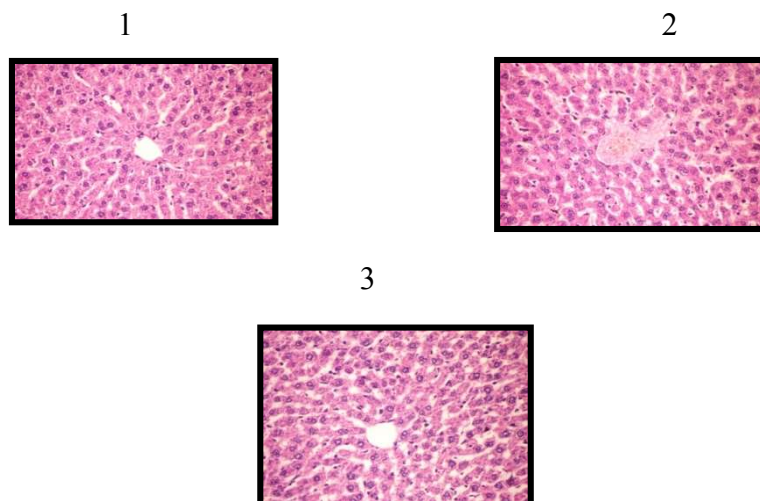
From table (3), it could be observed that administration of the formula 2 it is affect to rats activity (Group 3). The mixture of formula 2 induced significant increases in serum levels of immunological profile compared to control group. The other tested formula caused non significant changes in serum level of immunological productions.The main antioxidant compounds in formula 2 are vitamins C and E, phenolic compounds. So, different studies have shown that they have a protective antioxidant effect on immunity status, cancer and cardiovascular diseases(**Mallilinet al., 2008 and Murtyet al., 2010**)

Table(3):Effect of feeding 15% tested formula on immunological productions in normal rats.

Immunological Profile mg/dl	G1	G2	G3
IgE	59.87 ^b ±1.34	61.5 ^b ±0.12	65.17 ^a ±0.25
IgM	106.33 ^b ±3.5	108.65 ^b ±0.65	110.2 ^a ±0.005
IgA	106.5 ^b ±1.5	108.5 ^b ±0.5	113.1 ^a ±0.1
IgG	1089.66 ^c ±11.16	1095 ^b ±19.87	1101.05 ^a ±8.05

Values are mean±SD. Values in the same column sharing the same superscript letters are not statistically significantly different at (p<0.05)

Histopathological examination of liver of the negative control rats fed on basal diet revealed normal histological picture of hepatic lobule which consists of central vein surrounded by normal hepatocytes as shown in (photo. 1). Examination of liver of group (2) showed of hepatocytes and infiltration of leucocytes in hepatic sinusoid (photo. 2). Liver and the third mixture showed marked improvements with no observed pathological lesions (photo 3). These results were according to **Mallilinet al.(2008) and Murtyet al.(2010)** who found that tumericcan keep the liver tissue in normal status without any changes and improve the cells structure more than control group.



Photos (1): Histopathological changes detected in the liver of negative control, formula 1 and formula 2.

DISCUSSION

Several studies have showed that each of Soyproten have long been recognized as an excellent source of high-quality protein. The soyproten also contains a wide variety of chemical compounds that have potent bioactivity. Among these compounds are the isoflavones and the saponins. The goal of our research was to quantify isoflavone and saponin concentrations in elite soybean cultivars grown in different environments and to identify a naturally occurring high and low variety that could be used in animal studies of colon cancer. We observed significant environment \times genotype interactions for the cultivars and selected 2 that provided the range of concentration for isoflavones and saponins. These were grown in an adequate quantity for animal studies, which are ongoing. They explored the influence of isoflavones and saponins on human colon tumor cells in culture, Caco-2, to determine

potential mechanisms through which these compounds influence the carcinogenic process. We observed the inhibition of Caco-2 cell proliferation by isoflavones and saponins, suggesting a protective effect of these compounds in colon cancer. Using purified soy saponins, we found no negative effects on mouse growth, organ weights, or intestinal morphology when the diet contained up to 3% saponins by weight. Hence, soy isoflavones and saponins are likely to be protective of colon cancer and to be well tolerated. Continuing studies will explore the cancer-protective effects of these compounds in animal models (**Ruth et al., 2013**).

The insoluble carbohydrates in soyprotein consist of the complex polysaccharides cellulose, hemicellulose, and pectin. The majority of soybean carbohydrates can be classed as belonging to dietary fiber.

Within soybean oil or the lipid portion of the seed is contained the phytosterols: stigmasterol (17–21%), sitosterol(53–56%) and campesterol(20–23%) accounting for 2.5% of the lipid fraction.

Saponins, a class of natural surfactants (soaps), are sterols that are present naturally in a wide variety of food-plants: vegetables, legumes, and cereals—ranging from beans and spinach to tomatoes, potatoes and oats. Whole soybeans contain from 0.17 to 6.16% saponins, 0.35 to 2.3% in defatted soy flour and 0.06 to 1.9% in tofu. Legumes such as soybean and chickpeas are the major source of saponins in the human diet. Sources of non-dietary saponins include alfalfa, sunflower, herbs and barbasco. Recent studies have shown that saponins are potential functional food ingredients because of their physiological properties.

Soy contains isoflavones like genistein and daidzein. It also contains glycitein, an O-methylated isoflavone which accounts for 5–10% of the total isoflavones in soy food products. Glycitein is a phytoestrogen with weak estrogenic activity, comparable to that of the other soy isoflavones (**Teixeira et al., 2000**).

Effect of tested formula on immunological its effect on increasing antioxydantenzymes could be indirect result of their effect on lipids metabolism.

The histopathological results showed that rats supplemented with formula 2 can prevent/reduce diet induce fatty liver. This fat reduction in the liver was confirmed by serum lipid analysis and by measurement of liver specific marker enzymes as mentioned before (**Teunissen et al., 2013**).

On the basis of the present results, it could be conclude that formula 2 which contained 60 % Soy Protein , 20 % Hibiscus and 20% Turmeric especially at 15% may have synergistic effect and its intake of be useful for improving serum lipid profile, liver functions and immunological activity in rats. Moreover, this mixture has a promising effect on the liver tissues as it ameliorates the histopathological lesions seen in this organ of rats.

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دراسات بيولوجية لبعض التوليفات الغذائية على وظائف الكبد

د/ حنان سعيد عبد السلام حجران

قسم التغذية وعلوم الأطعمة - مدرس مادة بكلية التربية النوعية جامعة المنوفية

د/ نهاد رشاد الطحان

قسم التغذية وعلوم الاطعمة - كلية الاقتصاد المنزلي - جامعة المنوفية

الملخص:

هذه الدراسة تستهدف معرفة تأثير بعض التوليفات الغذائية على وظائف الكبد في الفئران. وقد تم تقسيم واحد وعشرين من ذكور الفئران البيضاء إلى (٣) مجموعات وسبعة فئران (٧) في كل مجموعة. وكانت المجموعة الأولى المجموعة الكنترول التي تتغذى على الوجبة الضابطة فقط كمجموعة كنترول سالبة. والمجموعات الاخرى تتغذى على الوجبة الضابطة التي تحتوي على ١٥% من الوجبة الاساسية التوليفة التي تحتوي على ٦٠% بروتين شرش اللين، و ٢٠% من الجزر و ٢٠% من الراوند والتوليفة (٢) تحتوي على ٦٠% بروتين الصويا، و ٢٠% الخبيزة و 20%. الكركم. تم تقييم وظائف الكبد عن طريق تقدير تركيز الإنزيمات من ترانسفيراز اسبارتاتي الأمينية (AST)، ألانين ترانسفيراز الأمينية (ALT)، الدهون (الكوليسترول والدهون الثلاثية)، الليبوبروتينات العالية والمنخفضة الكثافة. وأظهرت النتائج تحسنا نتيجة تناول التوليفة الغذائية رقم ٢ تليها التوليفة الغذائية رقم ١ على القياسات السابقة. ولذلك هذه الدراسة اظهرت أن وظائف الكبد في الفئران يمكن تحسينها بواسطة ١٥% من التوليفة الغذائية ٢ والتي تحتوي على ٦٠% بروتين الصويا، و ٢٠% الخبيزة و 20% الكركم.

مفتاح الكلمات : التوليفة الغذائية، وظائف الكبد - مكونات الكوليستيرول.