

Biological Studies of Some Nutritional Formula on Liver Functions

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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), cholesterol fraction (HDL-c, LDL-c, VLDL-c) and glucose. 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- glucose level.

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 most important medical herbs and is a kind of Asteraceae natively perennial grown in North America, which is used pharmacologically and for aesthetic enjoyment. In 2005, Turmeric products ranked among the top botanical supplements sold in the United States. Its root and subterranean stem were used by North America in early period to treat trauma and alleviate symptoms of infection and inflammation. The Turmeric have been proven to show good immune regulation, anti-inflammation and antioxidant capacity and with no hyper sensitivity or other side effects during clinical trial stages. Varieties of Turmeric all contain similar main ingredients including caffeic acid derivatives, alkamides, flavonoids, essential oils, and poly acetylenes, and medical activities of which are yet to be exactly identified with corresponding diseases. However, caffeic acid derivatives and alkamides have been proven to be active ingredients with immune regulation effects. Moreover, synergistic antioxidant effect of caffeic acid derivatives, alkamides and polysaccharide fractions was demonstrated by measuring their inhibition of *in vitro* Cu(II)-catalyzed oxidation of human low-density lipoprotein (LDL) (**Gulfranz et al., 2011**).

The Rhubarb root contain a high percentage of carbohydrate (total sugar from 44 to 88%), fat (0.2-0.5%), 15% salts and minerals, protein (2.3-5.6%), vitamins and a high percentage of dietary fiber (6.4-11.5%). The Rhubarb root contains 0.2-0.5% oil, whereas the seed contains from 7.7 to 9.7% oil. The fatty acids occur in both flesh and seed as a range of saturated and unsaturated acids, the seeds containing 14 types of fatty acids, but only eight of these fatty acids occur in very low concentration in the leaves. Unsaturated fatty acids include

palmitoleic, oleic, linoleic and linoleic acids. The oleic acid content of the seeds varies from 41.1 to 58.8%, which suggests that the seeds of Rhubarb root could be used as a source of oleic acid. There are at least 15 minerals in dates. The percentage of each mineral in dried Rhubarb root varies from 0.1 to 916 mg/100 gRhubarb root depending on the type of mineral. In many varieties, potassium can be found at a concentration as high as 0.9% in the flesh while it is as high as 0.5% in some seeds. Other minerals and salts that are found in various proportions include boron, calcium, cobalt, copper, fluorine, iron, magnesium, manganese, potassium, phosphorous, sodium and zinc. Additionally, the seeds contain aluminum, cadmium, chloride, lead and sculpture in various proportions. Rhubarb root contain elemental fluorine that is useful in protecting teeth against decay. Selenium, another element believed to help prevent cancer and important in immune function, is also found in dates. The protein in Rhubarb root contains 23 types of amino acids, some of which are not present in the most popular fruits such as oranges, apples and bananas. Rhubarb root contain at least six vitamins including a small amount of vitamin C, and vitamins B1 thiamine, B2 riboflavin, 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 (*Daucus carota* L.) contained a complex mixture of more than 100 compounds , some of which have not yet been identified or studied. A combination of volatile oils, fatty acids , flavonoids , saponins , proteins , and trace elements are believed to contribute to its effectiveness. It was found that both the oil and their active ingredients of the seeds, in particular thymoquinone (TQ), possess reproducible anti-oxidant effects through enhancing the oxidant scavenger system, which as a consequence lead to antitoxic effects induced by several insults (Dias, 2012). The effect of aqueous suspension of Carrot (*Daucus carota* L.) on carbon tetrachloride induced liver damage , CCL4 induced toxicity induced liver damage antagonize aqueous dose of 250-500 mg/kg suspension of Carrot (*Daucus carota* L.) by raising the level of LDH (Lactate dehydrogenase) and lowering of Carrot (*Daucus carota* L.) by raising the level of AST (aspartic transaminases) and ALT (L-alanine amino transfers) 5% seed of Carrot (*Daucus carota* L.) given to albino mice to evaluate hepatro 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 (*Daucus carota 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 (*Daucus carota 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 ± 5 g 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 ($25 \pm 5^\circ\text{C}$) 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°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 it, EDTA (ethylene diamine tetracetic acid) and centrifuged at 3000 r.p.m for 15 minutes. Serum was carefully aspirated and transferred into clean covet tubes 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 aminotransferase (AST) or (GOT) glutamic -oxaloacetic transaminase and glutamic pyruvic transaminase (GPT) or Alanine aminotransferase (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. Rats that were given formula 2 showed significantly higher levels of HDL-c compared to control group. 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, *Lee et al. (2009)* 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 ($p < 0.05$)
 3- **-Effect of feeding 15% tested formula on serum lipids on liver function enzymes 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 of feeding 15% tested formula on serum lipids on liver function enzymes 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 ($p < 0.05$)
 4--Effect of feeding 15% tested formula on serum lipids on immunological productions in normal rats .

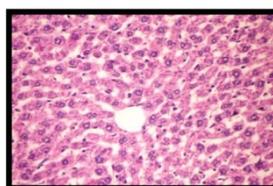
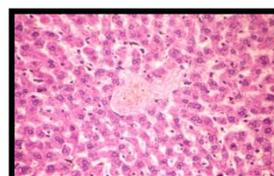
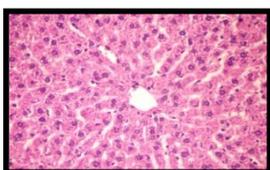
From table (3), it could be observed that administration of the formula 2 it is affect to rats activity (Group 3). The mixture of formula 2 induced significant increases in serum levels of immunological profile compared to control group. The other tested formula caused non significant changes in serum 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 (**Mallillin et al., 2008 and Murty et al., 2010**)

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

Immunological Profile mg/dl		G2	G3
IgE	59.87 ^b ±1.34	60.5 ^b ±0.2	64.17 ^a ±0.05
IgM	106.33 ^b ±3.5	106.65 ^b ±0.65	109.2 ^a ±0.005
IgA	106.5 ^b ±1.5	107.5 ^b ±0.5	111.1 ^a ±0.1
IgG	1089.66 ^c ±25.16	1089 ^b ±10.87	1100.05 ^a ±9.05

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 **Mallillin *et al.* (2008)** and **Murty *et al.* (2010)** who found that tumeric can 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 Soyprotien have long been recognized as an excellent source of high-quality protein. The soyprotien 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) Ingesting oligosaccharides such as raffinose and stachyose, namely, encouraging indigenous bifidobacteria in the colon against putrefactive bacteria.

The insoluble carbohydrates in soyprotien 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 antioxidant enzymes could be indirect result of their effect on lipid 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 concluded that formula 2 which contained 60 % Soy Protein , 20 % Hibiscus and 20% Turmeric especially at 15% may have synergistic effect and its intake could 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

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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 تحتوى على ٦٠٪ بروتين شرش اللبن ، و ٢٠٪ من الجزر و ٢٠٪ من الراوند والتوليفة (٢) تحتوي على ٦٠٪ بروتين الصويا، و ٢٠٪ الخبيزة و ٢٠٪ 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 تحتوى على ٦٠٪ بروتين الصويا، و ٢٠٪ الخبيزة و ٢٠٪ الكركم.

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