

Therapeutic and immunological effects of strawberry extracts in hepatic rats: a biological investigation.

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Abstract

Background: This study was aimed to determine the effects of different levels of strawberry extracts on biochemical and immunological changes of infected mice liver.

Methods: The study used (20) white Albino rats and were divided into two main groups first set of mice infected with hepatitis Second group of negative control, a non-mice group infected and was then the first and second main group. The selected groups were fed with different concentrations of strawberry extracts 5%, 10%, 15% and one group positive control infected with the disease which does not feed on the experimental diet and another negative control non-infected. The mice were then injected with carbon tetrachloride twice a week for two weeks.

Results: The present results demonstrated the best treatments in the level of total cholesterol and triglycerides and LDL appeared in the experimental diet groups, 10% and 15% of strawberry extracts and also showed the immunological results significantly differ in each experimental diet groups when compared to the negative control groups.

Rationale: These results recommend the hepatic patients to add strawberry extracts in their diet because it contains useful components.

Keywords: Strawberry extracts, Hepatitis, Immunological change.

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Introduction

Strawberries (*Fragaria*) have been known by different suffixes used for different varieties, such as *Fragaria vesca* for wild strawberry, and *Fragaria orientalis* for strawberries found in Siberia. Strawberries grow in bushes and are delicious seasonal fruits that also boost your [1] immune system. We know that fruits, particularly berries and those with exotic colors are rich in antioxidants, which means that they are huge boosters to your health. Strawberries are no exception to this rule. In addition to antioxidants, the strawberries possess polyphenols also [2]. The immune system is our body's first line of defense against infections, microbial activities, and a variety of other potentially dangerous conditions. Vitamin C, which is present in the strawberry, boosts the immune system and help in curing the common cough and cold. Vitamin C is also an antioxidant, which means that it neutralizes free radicals, the harmful byproducts of cellular metabolism that are being constantly created in our body. These free radicals are responsible for mutating the DNA of healthy cells into cancerous cells and are subsequently responsible for a number of diseases, including heart disease and various cancers. A single serving of strawberries has approximately 150% of your daily requirement of vitamin C, isn't that incredible [3].

In addition to vitamin C and phytochemicals, the strawberries are rich in iodine as well, which is very helpful for regulating

the proper functioning of the brain and nervous system. Potassium, which is found in significant quantities in strawberries, also has been linked to improved cognitive functions by increasing the blood flow to the brain. Research studies on students have shown that when potassium levels of high concentration are consumed; memory and recall abilities seem to be strengthened in tests. This is the reason why bananas and strawberries are considered as brain food. On the other hand; the grape (*Vitis uniferal*) polyphenols are known to be beneficial as free radical scavengers. Previous experiments demonstrated that molecules other than polyphenols, such as simple carbohydrate and organic acid, can acts as free radical inhibitors [4]. The liver is the largest solid organ in the body and also the largest gland of the body. Actually the liver has two different types of glands; a secretary gland which has a specialized structure that is designed to allow it to make and secrete bile into the bile ducts. It also is an endocrine gland since it makes and secretes chemicals directly into the blood that have effects on other organs of the body. Bile is a fluid that both aids in digestion and absorption of fats as well as carries waste products into the intestine. The liver weighs about three and a half pounds (1.6 kg). It measures on average, about 8 inches (20 cm) horizontally (across), and 6.5 inches (17 cm) vertically (down), and is 4.5 inches (12 cm) thick [5]. The aim of the present study was to check the effect of strawberry extracts on biochemical changes (such as serum lipids-liver

function-kidney function and immunological changes) in hepatic rats.

Materials and Methods

Materials

The studied samples were (strawberry extracts), were obtained from local market, Cairo, Egypt. Casein, vitamins, salts and cholesterol powder and pure fine chemical carbon tetrachloride were purchased from El-Gomhoria Company, Cairo, Egypt. Normal male albino rats were obtained from Research Institute of Ophthalmology Medical Analysis Department, Giza, Egypt.

Preparation of extracts

The strawberry fruits were purchased, washed and cleaned. After that the drying of the strawberry was carried out fruits in clean place under room temperature with good aeration for 7 d. After drying the strawberry fruits without exposure to heat or sun rays, were stored in polyethylene bags. All the moisture was removed without heating and the strawberries were kept until crushing. The 10 kg of strawberry gave approximately 1 kg powder extract (we used 30 kg strawberry). After crushing the mixtures were mixed with experiment diet.

Biological experiment

Animals: Twenty (20) adult male albino rats (Sprague Dawley strain) were used in the present investigation. The animals were obtained from the veterinary medicine institute, Cairo, Egypt. Each rat was housed in special cage under controlled condition every day. The animals were observed for external appearance, shape, color and distribution of hair and physical activity. All rats were fed one week on control diet before the beginning of the experiment for adaptation; the rats were weight tow is a week for 4 w. The diet was presented to rats in special covered cups to avoid food loss. All rats were provided with water by glass tubes through wire cage. The rats were fed a diet labium through the period of experiment. The final weight was recorded for organs weight calculation. All the experiments were conducted in biological manufactory of faculty of home Economic, Monofia University.

Experiment design

Twenty (20) adult male albino rats Sprague Dawely Strain of an average (100-120 g) and age (45 d) were used. These rats were divided into two major groups (hepatitis groups addition to negative control group) after that first group were divided into 4 sub-groups (3 group treatment: with 3 different concentrations 5%, 10%, 15% of Strawberry extract) and one group positive controls has the disease without treatment and second group are negative control. That means all rats divided into 5 groups 4 rats in each sub group and fed several diets for 4 w.

Preparation of hepatitis rats

Normal healthy adult male albino rats were injection by carbon tetrachloride twice weekly for two weeks, according to the method described elsewhere and then investigated level of Got and Gpt by random select to any rat to obtained sample blood serum, after positivism form infect rats were divided into 5 sub-groups 4 rats in each sub group.

First main group (16 rats): Hepatitis rats were divided into 4 sub-groups according to the following scheme and 4 rats in each subgroup.

Subgroup 1: fed on basal diet only as the control positive.

Subgroup 2: fed on basal diet containing 5% Strawberry extract.

Subgroup 3: fed on basal diet containing 10% Strawberry extract.

Subgroup 4: fed on basal diet containing 15% Strawberry extract.

The second main group (4 rats): Healthy rats fed on basal diet as the negative control.

Samples collection

Blood: At the end of the 4 w of experimental period; the animals were fasted for 12 h then the incisions were made in abdomen and blood samples were obtained from the portal vein into heparin zed centrifuge tubes. Plasma was separated by centrifugation at 4000 rpm for 10 min at room temperature and then kept in plastic vial stored under frozen conditions until analysis.

Organs: The organs (liver, kidney and spleen) were excised, rinsed in chilled saline solution, then blotted on filter paper, and weighted separately to calculate absolute and relative organs weight.

Biochemical analysis

The biochemical analysis was conducted following the earlier standard procedures as described below.

1-Determination of total cholesterol [6].

2-Determination of triglycerides [7].

3-Determination of HDL-cholesterol [8].

4-Determination of LDL cholesterol and VLDL cholesterol [9].

5-Determination of plasma albumin [10].

6-Determination of serum creatinine: [11].

7-Determination of serum aspartate amino transferase and alanine aniino transferase (AST and ALT): [12].

8-Determination of alkaline phosphatase (ALP) [13].

9-Measurements of immunological indices [14].

Statistical analysis

The statistical analysis was carried out following the Snedecor and Cochran [15] methods.

Results and Discussion

Biological results

Effect of different feed concentrations (5%, 10% and 15%) of strawberry extracts on biochemical changes on hepatitis albino rats: Table 1 shows the effect of feeding different concentration of strawberry extracts on serum total cholesterol, triglyceride, LDL, and HDL in normal and hepatitis rats after 4 w of feeding. Total cholesterol value in normal rats group was (162.54 ± 12.23 mg/dl). While hepatitis rats groups fed on basal and supplemented diets with different levels of strawberry extracts (positive control, 15%, 10%, and 5% strawberry extracts) showed serum total cholesterol values (166.25 ± 10.35, 140.45 ± 10.35, 155.30 ± 45.30, and 160.20 ± 10.35 mg/dl), respectively. These results showed that the best treatment was in the group obtained after feeding 15% strawberry extracts.

Serum triglyceride value in normal rats group was found (86.32 ± 10.41) mg/dl. While in hepatitis rats groups fed on basal and supplemented diets with different concentrations of strawberry extracts were (81.20 ± 8.45, 70.25 ± 11.24, 72.45 ± 6.25, and 85.48 ± 4.18 mg/dl), for (positive control, 15%, 10%, and 5% Strawberry extracts), respectively. The best treatment was noticed in group fed with 15% strawberry extracts.

Serum LDL value in normal rats group was (102.24 ± 4.30 mg/dl). While hepatitis rats groups fed on basal and supplemented diets with different levels of strawberry extracts (positive control, 15%, 10%, and 5% Strawberry extracts) showed serum LDL values (109.15 ± 8.15, 80.45 ± 7.45, 88.35 ± 6.20, and 91.25 ± 4.30 mg/dl), respectively. Significant differences were observed in the group of 5%, 10% and 15% strawberry extracts when compared with negative control.

Serum HDL in normal rats group was (72.65 ± 0.02 mg/dl). While hepatitis rats groups fed on basal and supplemented diets with different levels of strawberry extracts (positive control, 15%, 10%, and 5% strawberry extracts) showed serum HDL values (70.54 ± 6.12, 86.15 ± 3.68, 78.35 ± 3.28, and 69.35 ± 4.46 mg/dl), respectively. These results showed the best treatment in the group fed with 15% strawberry extracts. These results are in agreement with those reported by Arpita [16], who studied the effect of freeze-dried strawberry powder and found that it improves lipid profile and lipid peroxidation in women with metabolic syndrome. They found that freeze-dried strawberry powder (FSP), a concentrated source of strawberry polyphenolic flavonoids, fiber, and phytosterols is a novel dietary fruit supplement marketed by selected fruit growers and special promotion groups. Not enough scientific data is available on the health benefits of this product. Our study shows the potential role of FSP in lowering total and LDL-cholesterol, and lipid peroxidation in women with metabolic syndrome, and suggests the need for larger

controlled trials. The data presented in Table 2 illustrated effect of feeding different concentration (5%, 10% and 15%) of strawberry extracts on liver function of hepatitis rats.

Aspartate amino transaminase (AST) value in the control (-) was (19.22 ± 0.15 U/l). While hepatitis rats groups: fed on basal and supplemented diets with different levels of strawberry extracts (positive control, 15%, 10%, and 5% Strawberry extracts) showed serum AST values (225.25 ± 15.15, 105.35 ± 9.45, 155.25 ± 3.15 and 210.20 ± 1.10 U/L), 'respectively. Significant differences were noticed between all the groups of strawberry extracts when compared with negative control.

Alanine amino transaminase (ALT) value in control (-) was (31.77 ± 6.18 U/L). Hepatitis rats groups fed on basal and supplemented diets with different levels of strawberry extracts (positive control, 15%, 10%, and 5% Strawberry extracts) showed serum ALT values (305.46 ± 27.31, 175.45 ± 6.35, 225.25 ± 4.15, and 278.45 ± 4.45 U/L), respectively. Significant differences were observed between all groups of strawberry extracts when compared with negative control.

Alkaline phosphate ALP value in control (-) group was (96.45 ± 4.45 U/L). While hepatitis rats groups fed on basal and supplemented diets with different levels of strawberry extracts (positive control, 15%, 10%, and 5% strawberry extracts) showed serum ALP values (245.5 ± 35.20, 165.33 ± 8.90, 205.20 ± 8.25, and 230.05 ± 7.25 U/L), respectively. The results showed significant differences between all groups of strawberry extracts when compared with negative control.

Total bilirubin TBIN value in control (-) group was (0.55 ± 0.05 mg/dl). While hepatitis rats groups fed on basal and supplemented diets with different levels of strawberry extracts (positive control, 15%, 10%, and 5% strawberry extracts) showed serum TBIN values (3.25 ± 0.05, 2.45 ± 0.02, 2.65 ± 0.03, and 3.05 ± 0.04 mg/dl), respectively. The results confirmed significant differences between all groups of strawberry extracts when compared with negative control.

Albumin ALP values in control (-) group was (4.85 ± 0.23 g/dl). While hepatitis rats groups fed on basal and supplemented diets with different levels of strawberry extracts (positive control, 15%, 10%, and 5% strawberry extracts) showed serum TBIN values (1.06 ± 0.11, 1.85 ± 0.3, 1.25 ± 0.04, and 1.05 ± 0.20 g/dl), respectively. The results demonstrated significant differences between all groups of strawberry extracts when compared with negative control. Our result is in agreement with Sherifa et al. [17]. They aimed to evaluate the hepatoprotective effect of strawberry juice on experimentally induced liver injury in rats. To this end, rats were intraperitoneally injected with carbon tetrachloride (CCl₄) with or without strawberry juice supplementation for 12 w and the hepatoprotective effect of strawberry was assessed by measuring serum liver enzyme markers, hepatic tissue redox status and apoptotic markers with various techniques including biochemistry, ELISA, quantitative PCR assays and histochemistry. The hepatoprotective effect of the strawberry was evident by preventing CCl₄-induced increase in liver

enzymes levels. Determination of oxidative balance showed that strawberry treatment significantly blunted CCl_4 -induced increase in oxidative stress markers and decrease in enzymatic and non-enzymatic molecules in hepatic tissue. Furthermore, strawberry supplementation enhanced the anti-apoptotic protein, Bcl-2, and restrained the pro-apoptotic proteins Bax and caspase-3 with a marked reduction in collagen areas in hepatic tissue. These findings demonstrated that strawberry (*F. ananassa*) juice possessed antioxidant, anti-apoptotic and anti-fibrotic properties, probably mediated by the presence of polyphenols and flavonoid compounds. Data presented in Table 3 illustrated the effect of feeding different concentration of strawberry extracts on kidney functions in normal and hepatitis rats after 4 w of feeding. Creatinine and BUN values were (0.70 ± 0.02 and 10.12 ± 0.55 mg/dl) for the normal rats group. While hepatitis rats groups fed on basal and supplemented diets with different levels of strawberry extracts showed that creatinine levels were (3.55 ± 0.06 , 2.20 ± 0.03 , 2.65 ± 0.08 , and 3.15 ± 0.04 mg/dl) for (positive control, 15%, 10%, and 5% Strawberry extracts), respectively. The results proved significant differences between all the groups of WGS when compared with negative control.

BUN values for control (-) group was (10.12 ± 0.55 mg/dl). While hepatitis rats groups fed on basal and supplemented diets with different levels of strawberry extracts (positive control, 15%, 10%, and 5% strawberry extracts) showed serum BUN values (22.10 ± 0.25 , 12.40 ± 0.60 , 16.25 ± 0.20 , and 18.40 ± 0.10 mg/dl), respectively. These results depicted non-significant differences between all groups of strawberry extracts when compared with negative control. The present results are in the same line up. They showed that the effect of proanthocyanidin on rats which have renal ischemia-reperfusion. The increase of thiobarbituric acid (TBA) in kidney results in the alteration of activity of antioxidant enzyme (Dismutasecatalase and glutathione peroxides). They found that proanthocyanidin has protective effect against renal ischemia-reperfusion by reducing the level of (TBA). It also increased activity of antioxidant enzymes. Table 4 represent the effect of feeding different concentrations of strawberry extracts on serum ferritin, hemoglobin, hematocrite values in normal and hepatitis rats after 4 w of feeding.

Serum ferritin value in normal rats group was (48.2 ± 3.1 pg/L). While hepatitis rats groups fed on basal and supplemented diets with different levels of strawberry extracts (positive control, 15%, 10%, and 5% strawberry extracts) showed serum ferritin values (36.3 ± 2.25 , 44.9 ± 2.50 , 38.25 ± 0.03 , and 36.9 ± 4.45 pg/L), respectively. The results showed significant differences between all groups of strawberry extracts when compared with negative control.

Hemoglobin value in normal rats group was (11.92 ± 0.12 g/L). While in hepatitis rats groups fed on basal and supplemented diets with different levels of Strawberry extracts were (8.20 ± 0.11 , 10.95 ± 0.2 , 10.66 ± 0.25 , and 9.15 ± 0.33 g/L) for (positive control, 15%, 10%, and 5% Strawberry extracts), respectively. The results proved significant difference between all groups of strawberry extracts when compared with negative control.

Hematocrite value in normal rate group was ($37.25\% \pm 2.5\%$). While in hepatitis rats groups fed on basal and supplemented diets with different levels of strawberry extracts were (30.20 ± 2.2 , 35.20 ± 4.15 , 34.6 ± 2.2 , and $31.65 \pm 5.4\%$) for (positive control, 15%, 10%, and 5% strawberry extracts), respectively. The results demonstrated significant differences between 5%, 10% and 15% strawberry extracts when compared with negative control.

Immunological results

Table 5 represents the effect of feeding different concentration of strawberry extracts on immunity indices (serum IgG, serum IgM and total immunoglobulin) in normal and hepatitis rats after 4 w of feeding. Serum IgG value in normal rats group was (2850.5 ± 350.10 U/ml). While hepatitis rats groups fed on basal and supplemented diets with different levels of strawberry extracts (positive control, 15%, 10%, and 5% Strawberry extracts) showed serum IgG values (1500.7 ± 300.51 , 2540.9 ± 210.50 , 2350.60 ± 100.30 , and 1800.6 ± 250.2 U/ml), respectively. The results demonstrated non-significant differences between 5%, 10% and 15% strawberry extracts when compared with negative control.

The serum IgM value in normal rats group was (380.92 ± 15.12 U/ml). While in hepatitis rats groups fed on basal and supplemented diets with different levels of Strawberry extracts were (190.55 ± 10.24 , 295.40 ± 10.22 , 260.40 ± 45.30 , and 210.60 ± 15.50 U/ml), for (positive control, 15%, 10%, and 5% Strawberry extracts), respectively. The results demonstrated significant differences between 5%, 10% and 15% strawberry extracts when compared with negative control.

Total immunoglobulin value in normal rats group was (3650.25 ± 200.5 U/ml). While in hepatitis rats groups fed on basal and supplemented diets with different levels of strawberry extracts were (2232.78 ± 65.2 , 3000.45 ± 65.45 , 2700.50 ± 20.50 , and 2530.15 ± 60.4 U/ml), for (positive control, 15%, 10%, and 5% strawberry extracts), respectively. The results confirmed the significant differences between 10% and 15% strawberry extracts when compared with negative control.

Table 1. Effect of feeding different concentration of strawberry extracts on serum total cholesterol, triglyceride, LDL, and HDL on hepatitis albino rats.

Lipid parameters	15% extracts	Strawberry 10% extracts	Strawberry 5% extracts	Strawberry Control (+)	Control(-)
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Total cholesterol (mg/dl)	140.45 ± 10.35 ^a	155.30 ± 45.30 ^b	160.20 ± *0.35 ^b	166.25 ± 20.33 ^b	162.54 ± 12.23 ^b
Triglyceride (mg/dl)	70.25 ± 11.24 ^a	72.45 ± 6.25 ^b	85.48 ± 4.18 ^c	81.20 ± 8.45 ^c	86.32 ± 10.41 ^c
LDL (mg/dl)	80.45 ± 7.45 ^a	88.35 ± 6.20 ^a	91.25 ± 4.30 ^b	109.15 ± 8.15 ^c	102.24 ± 4.30 ^c
HDL (mg/dl)	86.15 ± 3.68 ^a	78.35 ± 3.28 ^b	69.35 ± 4.46 ^b	70.54 ± 6.12 ^b	72.65 ± 0.02 ^b

Total Cholesterol (Best= <200 mg/dL, Borderline high=200-239 mg/dL, High=240 mg/dL or higher) triglycerides (Best= <150 mg/dL, Borderline high=150-199 mg/dL, High=200-499 mg/dL, very high=500 mg/dl or higher) LDL cholesterol (Best= <100 mg/dL, Good=100-129 mg/dL, Borderline high=130-159 mg/dL, High=160-189 mg/dL, Very high=190 mg/dL or higher) HDL cholesterol (Low= <40 mg/dL, Best=60 mg/dL or higher) RGS; Red Grape Seed.

Table 2. Effect of feeding different concentration of strawberry extracts on liver functions of rats with carbon tetra chloride induced hepatitis.

Liver Function	15% Strawberry extracts	10% Strawberry extracts	5% Strawberry extracts	Control (+)	Control (-)
AST (IU/L)	105.35 ± 9.45 ^b	155.25 ± 3.15 ^c	210.20 ± r.10 ^d	225.25 ± 15.15 ^d	19.22 ± 0.15 ^a
ALT (IU/L)	175.45 ± 6.35 ^b	225.25 ± 4.15 ^c	278.45 ± 4.45 ^d	305.46 ± 27.31 ^d	31.77 ± 6.18 ^a
ALP (IU/L)	165.33 ± 8.90 ^b	205.20 ± 8.25 ^c	230.05 ± 7.25 ^d	245.5 ± 35.20 ^d	96.45 ± 4.45 ^a
TBIN (mg/dl)	2.45 ± 0.02 ^b	2.65 ± 0.03 ^b	3.05 ± 0.04 ^c	3.25 ± 0.05 ^c	0.55 ± 0.05 ^a
Alb (g/dl)	1.85 ± 0.30 ^b	1.25 ± 0.04 ^c	1.05 ± 0.20 ^d	1.06 ± 0.1 ^d	4.85 ± 0.23 ^a

Data presented as mean ± SE IU/L International Unit per Liter SGOT (Serum Glutamic-Oxaloacetic Transaminase)-Normal Range 10-34 IU/L SGPT (Serum Gamma Glutamyl Transpeptidase)-Normal Range 1-50 IU/L ALP (Alkaline Phosphatase)-Normal Range 44-147 IU/L TBIN (Total Bilirubin)-Normal Range 0.3-1.9 mg/dl Alb (Albumin)-Normal Range 3.4-5.4 g/dl

Table 3. Effect of feeding different concentration of Strawberry extracts on kidney functions of rats with carbon tetra chloride induced hepatitis.

Kidney Function	15% Strawberry extracts	10% Strawberry extracts	5% Strawberry extracts	Control (+)	Control(-)
Creatinine (mg/dl)	2.20 ± 0.03 ^b	2.65 ± 0.08 ^c	3.15 ± 0.03	3.55 ± 0.06 ^d	0.70 ± 0.02 ^a
BUN (mg/dl)	12.40 ± 0.60 ^b	16.25 ± 0.20 ^c	18.40 ± 0.10 ^c	22.10 ± 0.25 ^d	10.12 ± 0.55 ^a

Data presented as Mean ± SE Normal Range of Creatinine is 0.6-1.2 mg/dl BUN (Blood Urea Nitrogen) Normal Range 7-20 mg/dl

Table 4. Effect of feeding different concentration of Strawberry extracts on iron indices of rats with carbon tetra chloride induced hepatitis.

Tron indices	15% Strawberry extracts	10% Strawberry extracts	5% Strawberry extracts	Control (+)	Control (-)
Serum ferritin 0 ⁺ g/L)	44.9 ± 2.50 ^b	38.25 ± 0.03 ^c	36.9 ± 4/5 ^c	36.3 ± 2.25 ^c	48.2 ± 3.1 ^a
Hemoglobin (g/L)	10.95 ± 0.2 ^a	10.66 ± 0.25 ^b	9.15 ± 0.33 ^c	8.20 ± 0.1 ^c	11.92 ± 0.12 ^a
Hematocrite (%)	35.20 ± 4.15 ^b	34.60 ± 2.2 ^b	31.65 ± 5.4 ^c	30.20 ± 3.3 ^c	37.25 ± 2.5 ^a

Table 5. Effect of feeding different concentrations of Strawberry extracts on immunity indices of rats with carbon tetra chloride induced hepatitis.

Immunity indices	15% Strawberry extracts	10% Strawberry extracts	5% Strawberry extracts	Control (+)	Control(-)
IgG (U/ml)	2540.9 ± 210.50 ^b	2350.6 ± 100.30 ^b	1800.6 ± 250.20 ^c	1500.7 ± 300.51 ^d	2850.5 ± 350.10 ^a
IgM (U/ml)	295.40 ± 10.22 ^b	260.40 ± 45.30 ^c	210.60 ± 15.50 ^d	190.55 ± 10.24 ^d	380.92 ± 15.12 ^a
Total immunoglobulin (U/ml)	3000.45 ± 65.45 ^a	2700.50 ± 20.50 ^b	2530.15 ± 60.4 ^c	2232.78 ± 65.2 ^d	3650.25 ± 200.5 ^c

GPX (Glutathione Peroxidase GSH (Glutathione))

Conclusion

In summary our results strongly recommended to hepatic patients to add strawberry extracts in their diet because it

contains useful component that are supplemented with immunological properties.

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