Evaluation the biological and biochemical effects of Rhubarb and Hibiscus on health status by rats

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ABSTRACT:

Medicinal plants have been used at the suitable levels in healthcare since time immemorial. Studies have been carried out globally to verify their efficacy and some of the findings have led to the production of plant-based medicines. The present study was investigated the effects of Rhubarb, and Hibiscus on healthy status of rats. Twenty one male albino rats Sprague – Dawley were divided into (3) groups (7) rats in each group. The first group was control which fed on basal diet only as a control group. The second group was fed on basal diet containing 10% from rhubarb and the third group fed on basal diet containing 10% from hibiscus. Liver functions was assessed by estimation of plasma concentration of enzymes activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), lipid fraction (total cholesterol and triglyceride) and cholesterol fractions (HDL-c, LDL-c, VLDL-c) and determined total immunoglobulin (IgG, IgM, IgE and IgA). ***Result*** *showed that the hibiscus has a good effect on immunoglobulin test and showed increased in the liver enzymes, cholesterol fractions while decrease the level of HDL-c.* ***Conclusion:*** *This study concluded that using 10% from Hibiscus has a good effect on immunoglobulin in rats.*

Key words: *Liver enzymes - cholesterol fractions-rhubarb- Hibiscus*.

INTRODUCTION

Phototherapy's the treatment and prevention of diseases using plants or parts of it, such as leaves, flowers, roots, fruits, seeds, and rhizomes which it called medicinal plants, or medicinal 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 **(Govind and Madhuri, 2010)**.

The rhubarb root contain a high percentage of carbohydrate (total sugar from 44 to 88%), fat (0.2-/0.5%), 15% 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 fatty 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 linolenic 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. Rhubarb contain a lot of mineral in dried root varies from 0.1 to 916 mg/100g Rhubarb 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 that are found in various proportions include calcium, cobalt, copper, fluorine, iron, magnesium, manganese, potassium, phosphorous, sodium and zinc. Additionally, the seeds contain aluminum; cadmium and chloride 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. The protein in Rhubarb root contains a lot 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 pro of 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).**

Rhubarb is one of the lowest caloric vegetables and increases the rate at which the body burns fat. It can actually increase the levels of good cholesterol due to the presence of dietary fiber, which is known to scrape excess cholesterol from the walls of blood vessels and arteries. It has traditionally been used as a cure for constipation, but it was only recently discovered why it had such a powerful effect. By easing constipation and other digestive issues, you can prevent a wide range of more serious gastrointestinal disorders, including bloating, cramping, and even colorectal cancer. It can prevent the oxidation of brain cells and stimulate cognitive activity, thereby helping to delay or even prevent the onset of Alzheimer's disease and vitamin K also promotes osteotropic activity, meaning that it stimulates bone growth and repair. Combined with the rich amount of calcium and other minerals found in rhubarb, the vegetable as a whole is a major player in bone protection. The trace amounts of copper and iron found in rhubarb are enough to stimulate the production of new red blood cells, increasing the total RBC count in the body and increasing oxygenation of essential areas of the body, thereby improving their function and boosting the overall metabolism of the body. While, long-term treatment with rhubarb may result in bone loss,

weakness, potassium loss and heart rate irregularities. Rhubarb also contains high concentrations of oxalic acid, a chemical associated with the development of kidney stones. Excess dose of the plant about 9 grams for a 65-kilogram human led to the above side effect **(Gazanfar, 2016).**

Hibiscus 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 thymo-quinone (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)**. Hibiscus sabdariffa plant is antiseptic, diuretic, purgative, sedative and emollient. The leaves in combination with ginger are used to suppress high blood pressure and in treatment of hypertension. It can be used in making jams, jellies, ice cream, flavor and colorants in many drinks, a decoction of the leaves is taken as juice which helps in the improvement of health and immune system. Hibiscus sabdariffa leaves are used on sores and wounds. The ripe calyces, when boiled in water can be used to treat bilious attacks and cure ulcer and in combination with the flower can be used for tonic tea for digestive and kidney functions. In addition, the heated leaves are applied to cracks in the feet and on boils to speed maturation (**Haji and Haji, 2012). Onyenekwe et al., (2012)** found that it had the inhibition effect on the growth of unwanted pathogenic microorganisms like the Staphylococcus aureus, Escherichia coli, Aspergillusspp isolated. According to a study conducted among hyper-cholesterolemic patients, one capsule of Roselle extract (1 g), given three times a day (for a total of 3 g/day), significantly

lowered serum cholesterol, and reduced glucose while increasing high density lipoprotein cholesterol (Gurrola-**Diaz, et al 2014).**

Sun et al (2016) stated that aqueous suspension of Hibiscus exerts hepatic protective effect against hepatobiliary carcinogens because of their antioxidant property at the level 5% while, the excess of aqueous suspension of Hibiscus above this level led to low estrogen levels, reduce fertility in women, significantly affects skin and brain cancer cells, increases the risk of heart disease because it expands the blood vessels in the body .So, the present study was carried out to investigate biological effects of some nutritional formula contained hibiscus leaves and rhubarb root.

Aim of the study:

The present study was carried out to investigate biological effects of Hibiscus leaves and Rhubarb root on serum parameters (liver function, lipid profile and immunological productions) of rats.

MATERIALS &METHODS

Materials:

- The tested plants were obtained from local market in Cairo Governorate, Egypt.
- Chemicals and other materials: skimmed milk "protein", corn starch, DL-methionine, choline chloride, vitamins, and minerals were obtained from Morgan and El-gomhorya Company for Chemicals, Cairo, Egypt.
- Animals: Twenty one healthy adult male albino rats "Sprague Dawley strain" weighing 150± 5g obtained from the animal colony, Helwan Farm, Vaccine and Immunity Organization, Helwan Governorate, Egypt.
- Chemical reagents kits were purchased from Diamond Diagnostics (Egypt).

Methods:

Preparation of plant powder

Hibiscus leaves and Rhubarb root were washed and dried in drying oven at 50°C for 3days, then crushed and milled as a dried powder.

Animal's diet

- The standard diet prepared as described by **Reeves *et al*., (1993).**

Biological experiment:

Animals kept in single wire cages with wire bottoms under hygienic conditions (temperature 25 ± 5°C and light had a 12 h light-dark cycle). The diet introduced to the rats in special food containers which avoid scattering of food. Also, water provided to the rats by glass tube projection through the wire cages. Food and water will be provided ad-libitum and checked daily.

Experimental design

Twenty one male albino rats 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 ± 5g. Groups of rats were as the follows:

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

Group (2): Rats (n=7) were fed on basal diet containing 10% Rhubarb.

Group (3): Rats (n=7) were fed on basal diet containing 10% Hibiscus.

This level 10% of plant was added according to (**Banning, 2005)** who reported that the excess level of rhubarb and hibiscus was 10% from diet and this percent calculated from the percent of starch.

At the end of the experiment period (28 days), the animals sacrificed under ether anesthetized and blood samples collected in dry centrifuge tubes from hepatic portal vein. Serum separated by centrifugation of blood at 4000 rpm (round per minute) for 15 minutes at room temperature and kept in plastic vial at – 20°c till analysis.

Biological evaluation:

Feed intake (FI) and body weight gain (BWG):

During the experimental period (28 days), Animals and food were weighed twice a week. At the end of experiment feed intake and body weight gain calculated as a mean ± SE for each group.

BWG= Final weight (g) – Initial weight (g)/ Initial weight (g)

Feed efficiency ratio (FER):

Calculated as a follows:

FER = Body weight gain (g) / Feed intake (g)

Biochemical analysis:

Serum Alkaline phosphates (ALP) were determined according to the procedure of **(IFCC methods., 1983)**. Aspartate aminotransferase (AST) and 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-C was carried out according to the method of **(Fnedewaid1972)** and **(Gordonand Amer., 1977)**. The determination of VLDL-C (very low density lipoproteins) and LDL-C (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 examination:

Liver was removed by careful dissection, washed in saline solution (0.9%), dried using filter paper then weighed and the portion from which put in 10% formaldehyde to examine histopathology. 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 was carried out using the programme of Statistical Package for the Social Sciences (SPSS), PC statistical software (Version 11; Untitled–SPSS Data Editor). The results were expressed as mean ± standard error (mean ± SE). Data were analyzed using one way classification, analysis of variance (ANOVA). The differences between means were tested for significance using least significant difference (LSD) test at $p<0.05$. Independent T test was also used to determine the statistical difference between two means **(Sendcor and Cochran, 1979).**

RESULTS & DISCUSSION:

Data in table (1) showed the mean value of FI (g/day) of rats fed on control diet and tested diets. It could be observed that the mean value of FI and body weight gain of negative control group were higher than two groups which fed on formula contained rhubarb. There were significant differences between the tested formulas groups and negative control group; also there were significant differences between the group fed on formula with rhubarb and formula hibiscus for feed intake and body weight gain. For FER, there is no significant among all groups. These results harmony with finding obtained by **Münzbergová, (2012)** and **Gazanfar, (2016)** who showed that when rat fed on rhubarb in the diet cause low feed intake and body weight gain due to it had high percentage of dietary fiber (6.4-/11.5%) which gave the feeling of feeding up and it is one of the lowest caloric and increase the rate burns body fat.

The present findings in table (2) showed that diet which contains 10% of Hibiscus was the highest value in triglycerides

(TG) and VLDL-C while the lowest value in T. Cholesterol and LDL-C; results statically were significantly except T. Cholesterol value. The lowest value Serum HDL-C observed in group (2) fed diet with Rhubarb compared other groups. The decrease HDL-C level between group2 and group 3 was not significantly while it was significantly compared with control group. These results disagree with **Crouse et al., (1999)** who found that high percent of rhubarb can decrease LDL-C, total cholesterol and increase the level of HDL-C. While **Gulfraz et al., (2011)** reported that hibiscus reduce the absorption of lipids from diet. At the tested levels10% hibiscus **by Sun et al., (2016 and Gazanfar, (2016)** which was excess dose led to increase triglycerides, LDL and VLDL cholesterol and in the same time, it decreased the level of HDL-C

Data presented in table (3) showed that AST and ALT levels of group2 and group3 significantly increased when compared with the control group. There were no significant differences between groups 2 and 3. **Roberfroid, (2000)** reported that could be noticed that normal level crude fiber in tested plants is a group of indigestible carbohydrates. It can improve the function of the alimentary tract and also lower cholesterol levels and liver functions**.** While, **(Gazanfar,2016)** found that the increasing of Rhubarb powder intake led to increase the levels of liver enzymes when compared to normal level and increase the accumulation of fat in liver. Also, **Sun et al .(2016)** stated that the high level for long period of Hibiscus powder led to increase the levels of liver enzymes by increasing the building up fat and increasing the risk of liver cirrhosis, liver cancer or liver failure .

From table (4), it could be observed that administration of diet that contains 10% of Hibiscus **(G3)** was significantly affected on serum level of immunological productions. The diet that contains 10% of hibiscus induced significant

increases in serum levels of immunological profile compared to control group. While the other tested of diet10% of Rhubarb caused no significant changes in serum level of immunological productions. The main antioxidant compounds of diet that contain 10% of hibiscus 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 while excess of Hibiscus powder led to increase the immunity productions which causes after the consumption for a long time cancer, skin allergic and heart diseases **(Mallillin et al., 2008)**

Histopathological examination:

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 (photo1). Examination of liver of group (2) showed hepatocytes and infiltration of leucocytes in hepatic sinusoid (photo 2). Liver and the third mixture showed congestion of hepatic-portal blood vessel associated with necrosis of hepatocytes (photo 3). These results were disagreement with **Mallillin et al., (2008)** who found that rhubarb can keep the liver tissue in normal status without any changes and improve the cells structure more than control group while, **Gazanfar, (2016)** stated that dilatation and congestion of central vein and hepatic sinusoids . The histopathological results of these study 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 and vain, 2013).** Whereas, **Dias, (2012)** showed that excess dose of this plant for long time led to congestion of central vein and hydropic degeneration of some hepatocytes

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Table (1): Effect of feeding 10% tested formula on food intake, body weight gain and feed efficiency ratio in normal rats.

Variables	G1	G2	G3
	Mean ± S.D	Mean ± S.D	Mean ± S.D
FI (g/day)	12.27[a] ±0.13	4.56 [c]±0.20	9.00 [b]±0.39
BWG (g/28 days)	38.33[a]±3.05	15[c]±3.15	29.6[b]±5.50
FER	0.112 [a]±0.043	0.117[a]± 0.002	0.117[a]±0.003

Values are mean ± SD

Values in the same row with different litters are significantly ($p \leq 0.05$) different.

Table (2): Effect of feeding Rhubarb and Hibiscus on serum lipids in normal rats

Serum lipids	G1	G2	G3
Triglycerides	76.48^{b} ±0.13	76.8^{b} 1.03±	**81.4^{a} ±3.01**
Total cholesterol	89.44^{a} ±2.19	88.17^{a} ±3.15	**87.69^{a} ±1.13**
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	**15.3^{b} ±1.17**	**15.4^{a} ±4.34**	**16.3^{b} ±0.74**

Values are mean ± SD.

Values in the same raw sharing the same superscript letters are not statistically significantly different at ($p<0.05$)

Table (3): -Effect of feeding Rhubarb and Hibiscus on liver 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 raw sharing the same superscript letters are not statistically significantly different at ($p<0.05$)

Table (4): Effect of feeding Rhubarb and hibiscus on immunological productions in normal rats

Immunological Profile mg/dl	G1	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 raw sharing the same superscript letters are not statistically significantly different at ($p<0.05$)

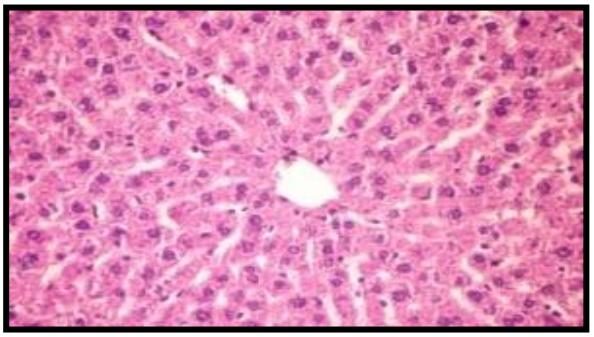

Photo (1): Liver of rats from control (-) group showing the normal histology of hepatic lobule (H and E×200).

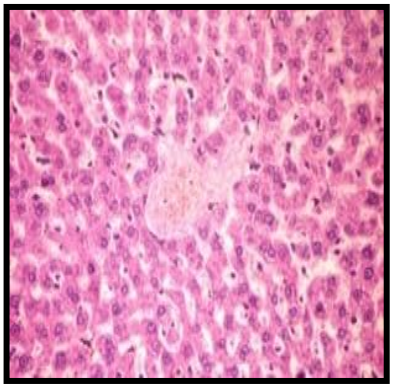

Photo (2): Liver of rats from group 2 showing dilatation and congestion of central vein and hepatic sinusoids (H and E×200).

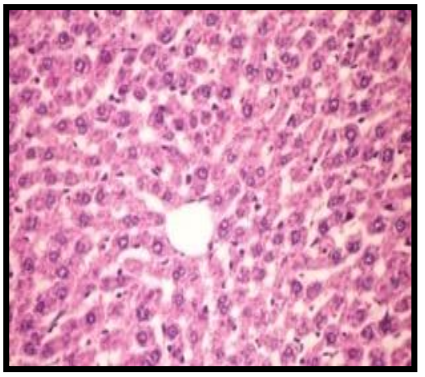

Photo (3): Liver of rats from group 3 showing congestion of hepatoportal blood vessel associated with necrosis of hepatocytes(H and E×200

تقييم التأثير البيولوجي والبيوكميائي للراوند والكركديه علي الحاله الصحيه للجرزان

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المعهد القومي للتغذية– القاهرة - مصر

الملخص العربي

انتشر استخدام النباتات الطبية في المجالات الطبية لما لها تأثير بالغ الأهمية في علاج العديد من الإمراض. تهدف هذه الدراسة إلى دراسة أثار الراوند والكركديه علي الوضع الصحي للفئران السليمة وفى هذه الدراسة تم استخدام 21 فأر من فئران االالبينو البالغة مقسمه إلي 3 مجموعات تحتوي كل مجموعه علي 7 فئران ،المجموعة الأولي هي المجموعة الضابطه والتي تم تغذيتها علي الوجبه الأساسية آما المجموعة الثانية تم تغذيتها علي الوجبه الأساسيه مع احتوائها علي 10% من الراوند والمجموعة الثالثة تم تغذيتها علي الوجبه الأساسية مع احتوائها علي 10% من الكركديه.وفى نهاية التجربة تم تقييم وظائف الكبد وكذلك تحليل دهون الدم والذي يشمل الكوليستيرول الكلي والدهون الثلاثية والكوليستيرول المرتبط بالبروتين مثل (الكوليستيرول عالي الكثافه والكوليستيرول منخفض الكثافه والكوليستيرول المنخفض الكثافه جدا) و تقييم الحاله المناعيه وكذلك فحص الانسجه التحاليل الهستولوجيه للانسجه. اظهرت النتائج زيادة في انزيمات الكبد الكوليستيرول منخفض الكثافه والكوليستيرول المنخفض الكثافه جدا والدهون الثلاثي نتيجة لتناول 10% من الكركدية والراوند كذلك ظهر خلل في الانسجة نتيجة للفحص الميكرسكوبي ، بل التحسن كان واضحا في الحاله المناعيه .

الكلمات المفتاحية: *التقيم البيولوجي والبيوكيميائي- الكركدية - الراوند*