

Synthesis, Characterization and Antihyperlipidemic of Rutin-calcium (II) Complex

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Abstract: A rutin-ca complex was synthesised in methanol and was characterized using elemental analysis, UV-Vis spectroscopy, IR spectroscopy and differential thermal analysis. Spectroscopic data suggested that the metal/ligand ratio of the complex is 2:1. The antihyperlipidemic activity of the complex was evaluated by animal experiment of 30 wistar rats. It was shown that the complex was much more effective antihyperlipidemics than the free flavonoid.

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1. Introduction

Flavonoids is a group of natural polyphenolic compounds that widely distributed in plant foods, which shows a wide range of biological activities and pharmacological properties, such as cardiovascular protection, anti-cancer, anti-ulcer, anti-virus and anti-inflammatory [1]; it is an kind of important natural antioxidant, free radical scavenger as well as metal chelator [2]. Rutin (quercetin-3-O-rutinoside) is the most common flavonol widely distributed in higher plants, such as vegetables, fruits, especially citrus fruits (oranges, grapefruit, lemon), buckwheat and beverages. The most important properties of rutin is scavenging

oxygen free radicals, such as hydroxyl radicals, oxygen radicals and peroxy radicals [3]. The ability of scavenging free radicals of rutin have been demonstrated by vitro experiments [4]. what's more, rutin has been used in the treatment of diseases [5]. The pharmacological activity of rutin including: anti-allergic[6], anti-inflammatory and dilation of blood vessels[7], anti-cancer [8], anti-bacterial [9], anti-virus [10]. Clinically for the treatment of allergic purpura and a variety of increased capillary fragility caused by bleeding disorders [11], also used to treat high blood pressure and senile bronchitis[12].

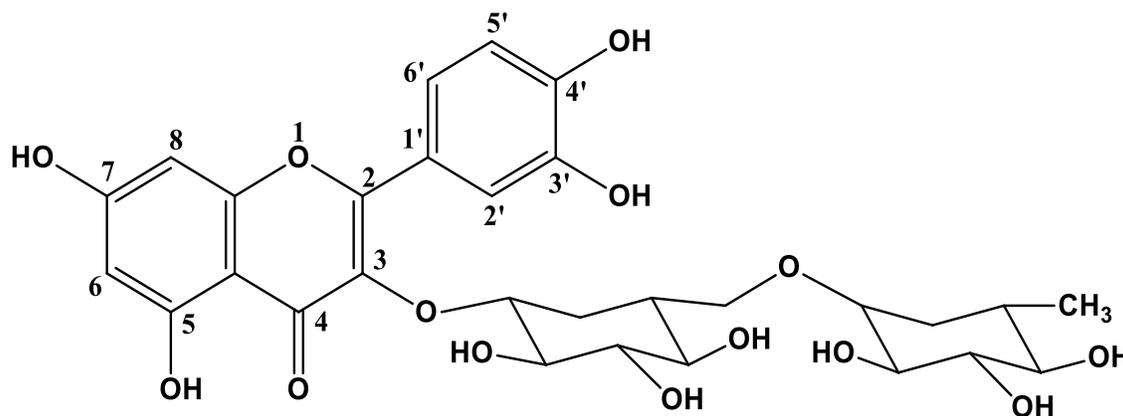


Fig.1 The chemical structure of rutin

Rutin, which has the appropriate spatial configuration and strong ligand oxygen atoms, is an excellent metal chelator [13], can form the stable complexes with many metal ions. Studies show that the biological activities of rutin complexes is stronger than that of the ligand [14]. The study on rutin metal complexes has aroused widespread concern [15]. So far, many a rutin complex has been reported: Al (III), Zn (II) [16], Cu (II) [17], Co (II) [18], Ni [19], Fe (II) [20], Fe (III) [21], Mn (II) [22], V (II) [23], U (II) [24], Au (III) [25]. In daily life, regular supplementing of rutin-rich foods, such as ginkgo biloba, buckwheat, tomatoes, onions, tea, may be contributed to scavenging free radicals and reducing the content of heavy metal ions in our body [26].

Calcium is one of the most important elements in living organisms. It plays an important role in the metabolism of nitrogen in plants, and represents the structural component of animal bones [27]. In cell physiological level, calcium is used to regulate the permeability and electrical properties of biological membranes, which in turn function as a signal for many cellular processes [28]. Calcium ion also serves important functions in blood clotting, and by acting as a "second messenger" in signal transduction, in the triggering of muscle contractions, and in the

transmissions of nerve impulses [29]. In addition, it can lower the levels of α -LP and β -LP. The atherosclerosis and cardiac arrhythmia could be induced by the lack of calcium [30]. It is suggested that the biological activity of an organic ligand can be increased when coordinated or mixed with suitable metal ion; because of its ability to act as free radical acceptor [31].

Herein, we put forward the methodology of the complexation process between Ca (II) and rutin, characterization of the complex as well as the investigation of antihyperlipidemic properties of the rutin-calcium complex.

2. Materials and methods

2.1 Reagents

Rutin was purchased from Sigma (Sigma Chemical Co., St. Louis, MO, USA). The other reagent and solvents were of analytical reagent grade. Cholesterol was purchased from Shanghai Chemical Reagent Company, sodium cholate from ASUS Shanghai Fine Chemicals Company, propylthiouracil from Shanghai Fuxing Zhaohui Pharmaceutical Co., Ltd., total cholesterol kit, triglyceride kit and high-density lipoprotein cholesterol kit from BIOSINO Biotechnology Co., Ltd., and thirty healthy Wistar rats weighing 200 ± 12 g from the experimental animal center of Henan province.

2.2 Instrumentation

UV Spectra was obtained by a UV-2550 ultraviolet-visible spectrophotometer using standard 1.00cm quartz cells (Shimadzu Corporation). Elemental analyses were performed using Perkin-400CHN elemental analyzer. The IR spectral was recorded by using KBr pellets in the spectral range 4000-400 on a FTS-3000 FTIR spectrophotometer. Melting points were determined on a X-5 melting point apparatus and are uncorrected. The differential thermal analysis of the complex was performed by the Thermal analyzer STA 409 PC/PG (Netzsch, Germany), from room temperature to 784 °C at a heating rate of 10°C/min.

2.3 Synthesis of the complex

Rutin (0.61g, 1mmol) was added to a 100ml round-bottom flask containing 25ml MeOH, stir 15min until the solid rutin was completely dissolved, then the pH value was adjusted to 8.6 with CH₃ONa. Anhydrous calcium chloride (222mg, 2mmol) dissolved in methanol (20ml) was added. The reaction mixture was refluxed for 4 h and monitored by TLC. The formed complex was collected by filtration and washed three times with a 1:3 methanol/water mixture and then several times with water and dried in a vacuum desiccator. A brown-yellow solid rutin-calcium complex was then obtained (m.p:301-305°C).

2.4 Antihyperlipidemia activity of the complex

2.4.1 Method

The preparation of fat emulsions: In a 500ml beaker, 80g of lard were heated to melt. 40g of cholesterol were added and mixed well. 4g of propylthiouracil and 8g of sodium cholate are mixed in a mortar. Then the powder, 80ml of tween 80, 80ml of 1, 2-propanediol was added into the mixture and was stirred until smooth. And double-distill water was added to 400ml. The emulsions were stored in the

refrigerator. Before application, it needed to be heated. The concentration of the fat emulsions was: cholesterol 100g/L, lard 200g/L, sodium cholate 20g/L, propylthiouracil 10g/L.

30 Wistar rats were randomly divided into three groups: normal control group, model group and experimental group, and were fed with common feedstuff. The rats in the normal control group were given normal saline [10ml/(kg·d)] for 14 days. The rats of the model group were administered intragastrically with fat emulsion [10ml/(kg·d)] for 14 days while the rats of the experimental group were administered intragastrically with fat emulsion and rutin-calcium complex [300mg/(kg·d)].

After continuous intragastric infusion for 14 days, the serum TC, TG, HDL was measured respectively. All rats were sacrificed to weigh the liver, heart, spleen and kidney and to compute the organ coefficient.

2.4.2 Statistical treatment

SPSS 10.0 software was used for homogeneity of variance and one-way ANOVA. Then LSD-t test was used for multiple comparison. The associations of the serum lipid levels and Viscera Index were analyzed by using Pearson correlation analysis.

3. Results and discussions

In this study, fifteen experiments that we designed were performed from pH 7.2 to pH 10 with every 0.2 pH as an experimental unit. The results showed that the optimum pH is 8.6. In the same way, we found that the optimum reaction temperature was 85°C and the optimum reaction time was 4h.

3.1 elemental analysis

The chemical compositions were analyzed by the combustion analysis spectrum for C, H and EDTA titration was applied to the determination of calcium ion. The results are listed in table 1.

Table.1. Elemental analysis of the rutin-ca complex

Element	The contents of elements (%)	
	Found values	Calculated values
C	46.28	47.40
H	5.31	5.27
Ca ²⁺	2.93	3.02

We know that coordination molar ratio of rutin to calcium is 1:2.

3.2 UV/Visible spectrum analysis

The UV/vis spectrum of the rutin-ca complex and rutin in MeOH are described in Fig.2 and 4. Rutin, like most flavones, exhibited two major absorption bands in

the UV/vis region, namely 355nm (band I) representing B-ring absorption (cinnamoyl system), and 257nm (band II) representing the A- ring absorption (benzoyl system).

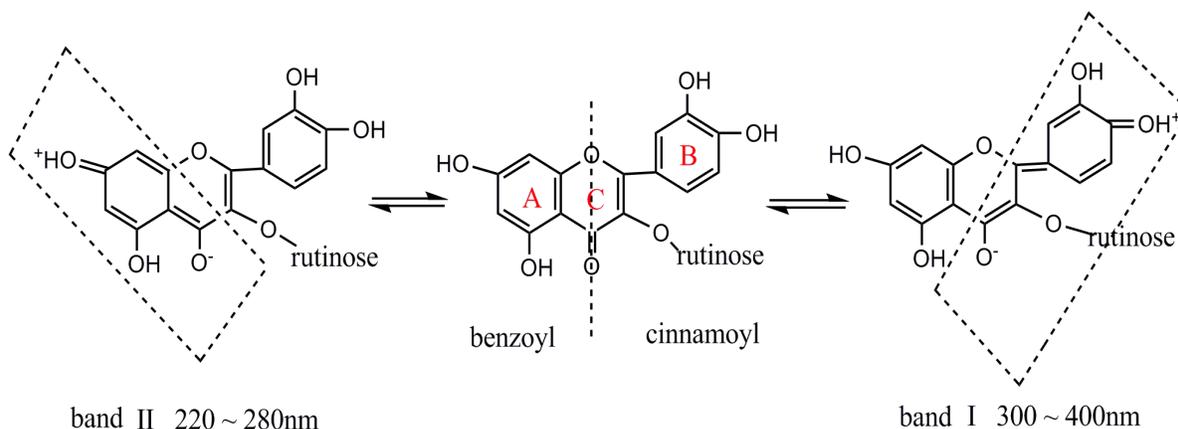


Fig.2. The structure of rutin and the division of band I and band II related to UV/vis absorption bands

The spectra were related to the $\pi-\pi^*$ transitions within the aromatic ring of the ligand molecules. In comparison with flavonoids absorption spectra, the

band of the complex was shifted to the long-wavelength region as shown in Fig.3.

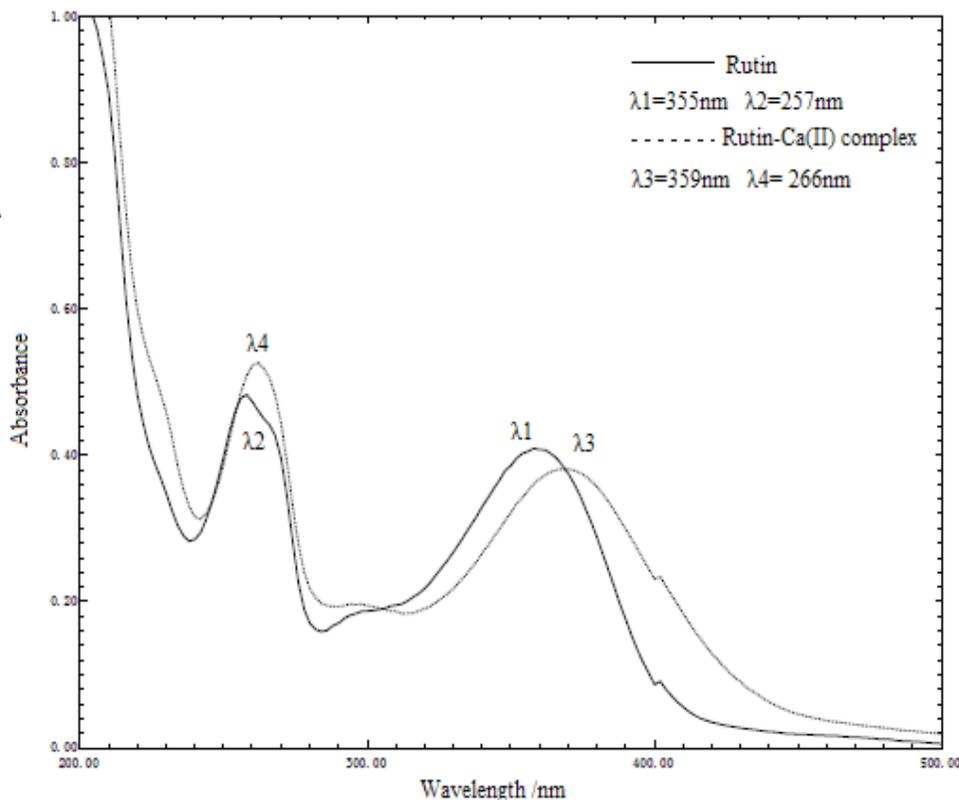


Fig.3. UV-visible absorption spectrum of rutin calcium complex

The isosbestic point which was characteristic of the formation of a complex was observed at 359nm. Such bathochromic shift could be explained by the extension of the conjugated system with the complexation. After chelating Ca (II) ion, each of the two bands had a shift (from 355 to 359nm for band I

and from 257 to 266nm for band II)[32].

3.3 IR spectroscopy analysis

IR spectroscopies of the rutin-ca complex and rutin were analyzed and the main data are summarized in Table 2.

Table.2. IR spectra data (cm-1) of the rutin-ca complex and rutin

Compound	ν (O-H)	ν (C=O)	ν (C=C)	ν (C-OH)	ν (C-O-C)
Rutin	3423.42	1655.03	1603.69	1361.52	1295.67
Ca-rutin	3377.50	1632.45	1551.29	1355.81	1288.76

The spectra presented evidence for the coordination between the metal ions and flavonoid molecules. Some features of the spectra are shown in Fig.4 and 5.

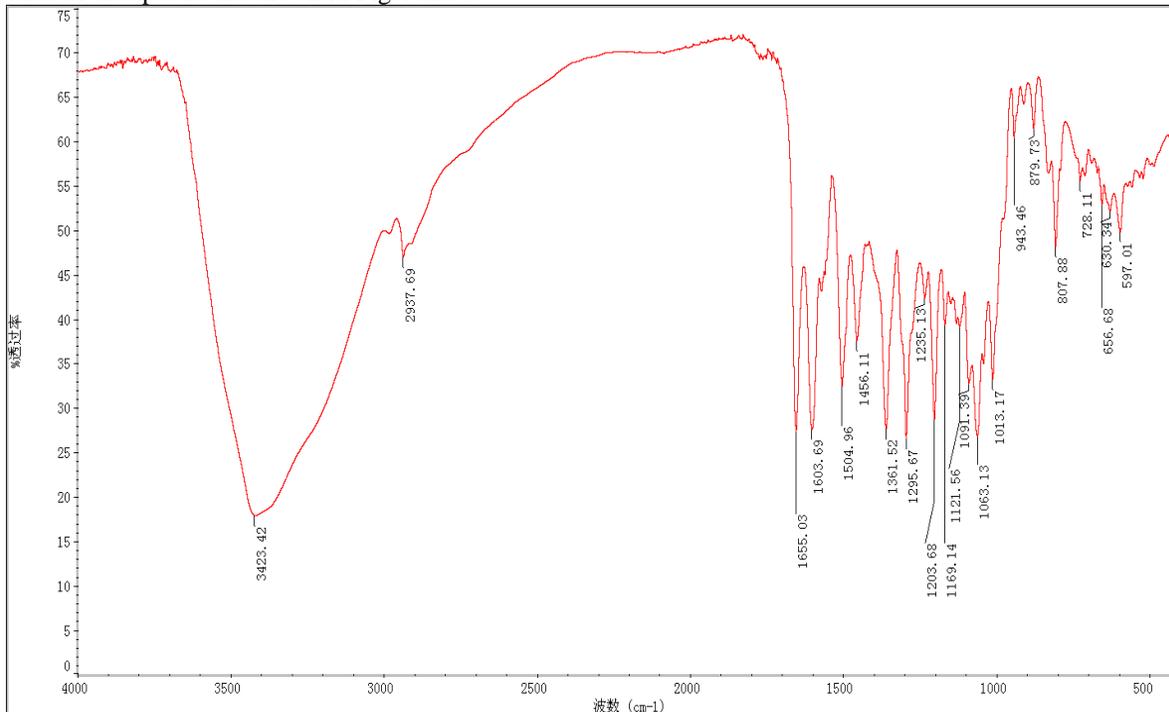


Fig.4. IR spectrum of rutin

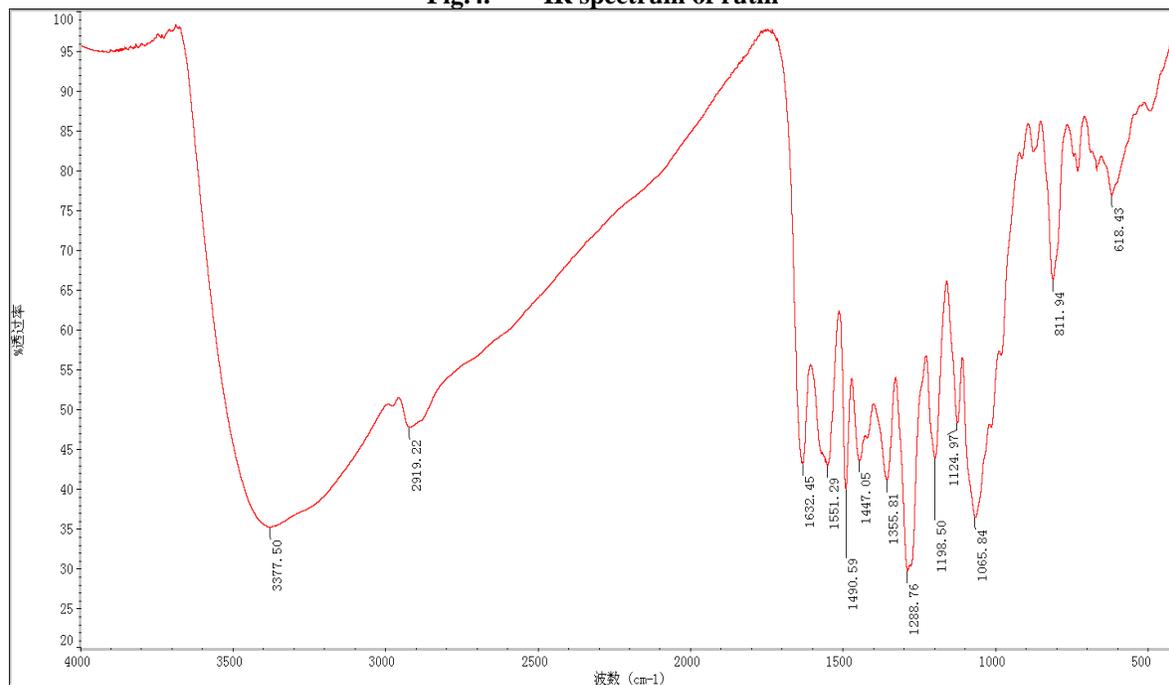


Fig.5. IR spectrum of the rutin-ca complex

The characteristic stretching $\nu(\text{C}=\text{O})$ mode of the rutin occurred at 1655.05 cm^{-1} , while due to the formation of the rutin-ca complex, this band shifted to 1632.45 cm^{-1} . It suggested that the Ca (II) coordination occurred through the carbonyl oxygen atom and the 5-OH group of the rutin. The shift of $\nu(\text{C}-\text{O}-\text{C})$ vibration frequency was much slighter (6.91 cm^{-1} from 1295.67 cm^{-1} of rutin to 1288.76 cm^{-1} of rutin-Ca(II) complexes) than that of $\nu(\text{C}=\text{O})$ vibration frequency (obviously declines by 22.58 cm^{-1}) indicating that the ring oxygen was not involved in the complexation. There was also a decline of 52.40 cm^{-1} for the $\nu(\text{C}=\text{C})$ vibration frequency because the conjugative effect was obviously enhanced after the chelation occurring [33]. The big bound of $\nu(\text{O}-\text{H})$ vibration frequency (from 3423.42 to 3377.50 cm^{-1}) indicated the existence of water in the complex [34].

3.4 ^1H NMR spectroscopy analysis

The ^1H NMR (400 MHz, DMSO) data of rutin and its complex with Ca(II) are given below separately:

Rutin: 12.630 (5-OH), 10.831 (7-OH), 9.646 (4'-OH), 9.186(3'-OH), 7.55 (2'-H, 5'-H, 6'-H), 6.836(8-H), 6.388(6-H) [35].

Rutin-Ca complex: 10.306(7-OH), 9.049 (3'-OH), 7.520(2'-H), 7.404(6'-H), 7.294 (5'-H), 6.470(8-H), 6.315(6-H).

The ^1H NMR data shows that the δ values of rutin-ca complex were shifted to higher field compared with the pure rutin. It maybe due to the increase of the conjugation caused by the coordination. The absence of 5-OH and 4'-OH reveals that they were involved in the coordination when the complex is formed [36].

Table 2 ^1H NMR (cm^{-1}) of rutin and rutin-Ca (II) complex

	Chemical shift (ppm)								
	5-OH	7-OH	4'-OH	3'-OH	2'-H	6'-H	5'-H	8-H	6-H
Rutin	12.631	10.831	9.646	9.186	7.590	7.561	7.501	6.836	6.388
Rutin-Ca		10.306		9.049	7.520	7.404	7.294	6.470	6.315

3.5 The differential thermal analysis

Thermal methods of analysis provided interesting ways for the investigation of metal complexes. Heating of material caused occurrence of chemical and physical transformations, which were accompanied by the absorption or liberation of heat [37]. The stability and structure of the complex could be determined by the thermogravimetric analysis [38]. Table 3 showed the maximum temperature values for decomposition along with the corresponding weight loss values. Thermogravimetric analysis was carried out for rutin-Ca (II) complex under the flow of air. Fig.7 showed the characteristic thermal events those change with heating

rate of $10^\circ\text{C}/\text{min}$ and the temperature range was from room temperature to 784°C .

Thermal analysis of the rutin-Ca (II) complex showed two distinct breaks i.e. dehydration and decomposition. It was found the weight loss was 4.83% below 77°C which might be attributed to the liberation of two moles of hydrated water molecules. When the complex was heated to 182°C , the weight loss was 9.12% which might be due to loss of the four coordinated water molecules. This fact suggested that the rutin-Ca complex contains four coordinated water molecules and two crystal water molecules.

Table 3 The maximum temperature, $T_{\text{max}}(^\circ\text{C})$, and weight loss values of the decomposition stages for the rutin-Ca complex

Compound	Mass(g/mol)	Decomposition $T_{\text{max}}(^\circ\text{C})$	Eliminated species	%mass loss	
				Found	calculated
[Ca ₂ (L)(H ₂ O) ₄]-2H ₂ O (795.66g/mol)	First step	82	2H ₂ O	4.83	4.52
	Second step	182	4H ₂ O	9.12	9.05
	Third step	350	C ₁₂ H ₁₀ O ₄	29.45	27.40
	Fourth step	570	C ₁₂ H ₂₁ O ₉	36.66	38.83
	Fifth step	720	C ₂ O	5.69	5.03
	Residue			CaO	14.25

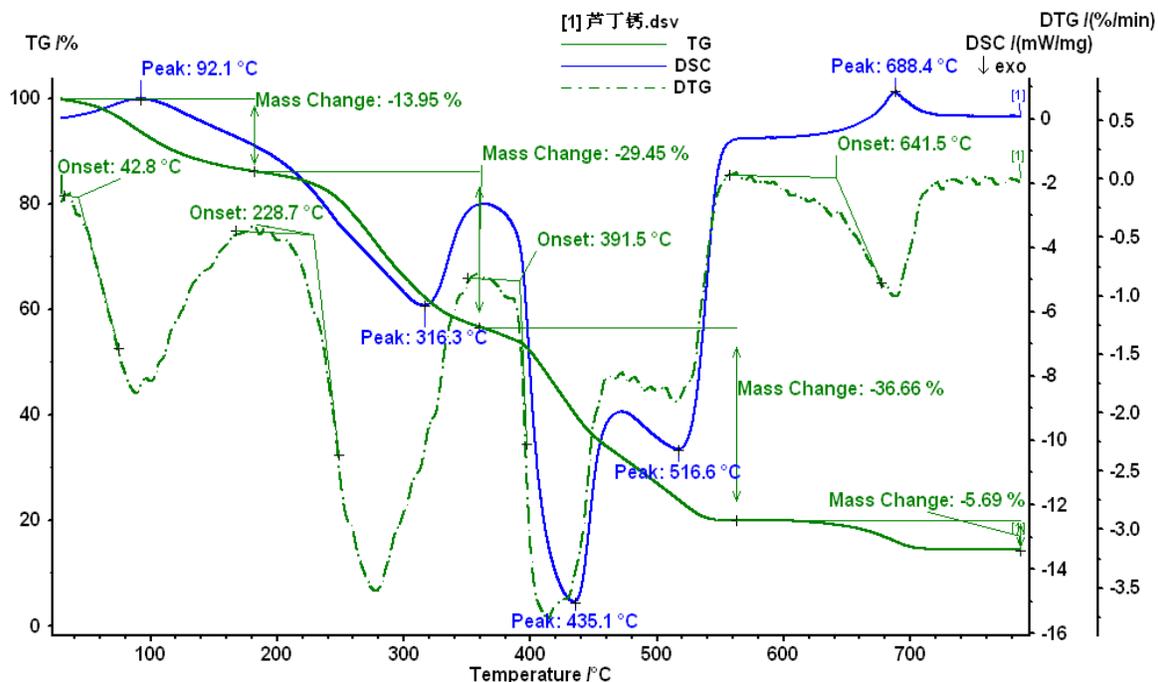


Fig.6 Thermogravimetric analysis of rutin-Ca complex

The DSC for the complex showed endothermic and exothermic peaks that agreed with the mass losses observed in the TG. The endothermic peaks about 92.1°C are due to dehydration. The exothermic peak about 316.3°C is due to the elimination of pyrocatechol (Fig.7. Part a) and the weight loss at this

step is 29.45%. The second exothermic peak around 435.1°C may be associated with the oxidative degradation of the remaining organic component (Fig.7. Part b), corresponding to 36.66% weight loss. The final products are the metal oxides.

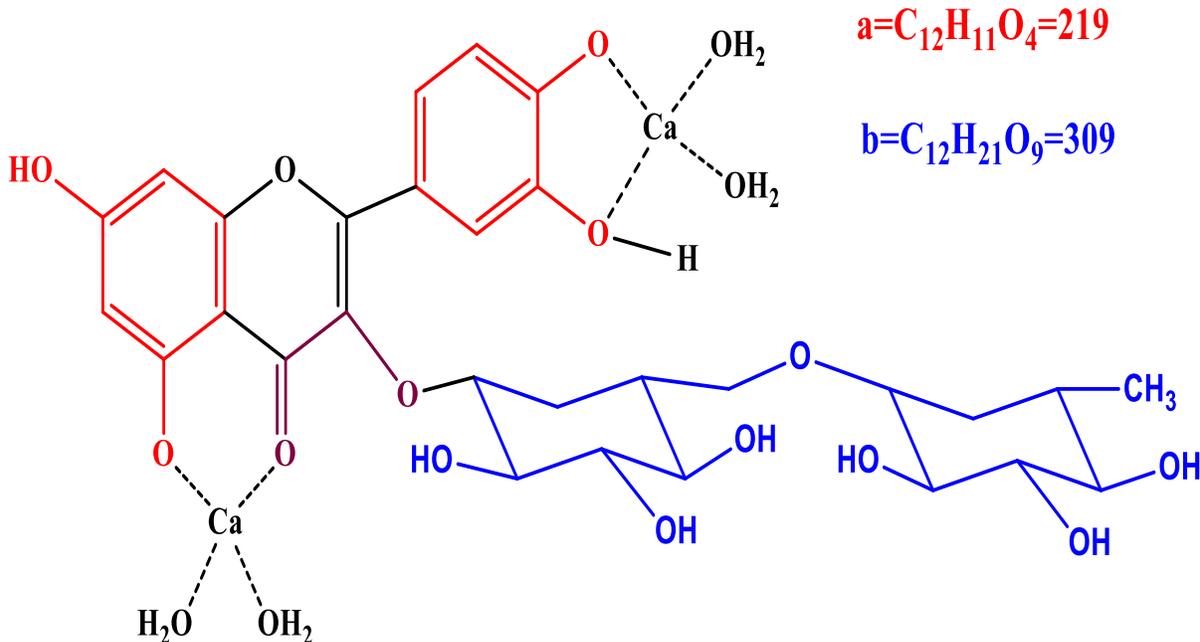


Fig.7. Schematic of Rutin-Ca complex thermal cracking

From this data, we could infer the structure of rutin calcium complex (Fig.8):

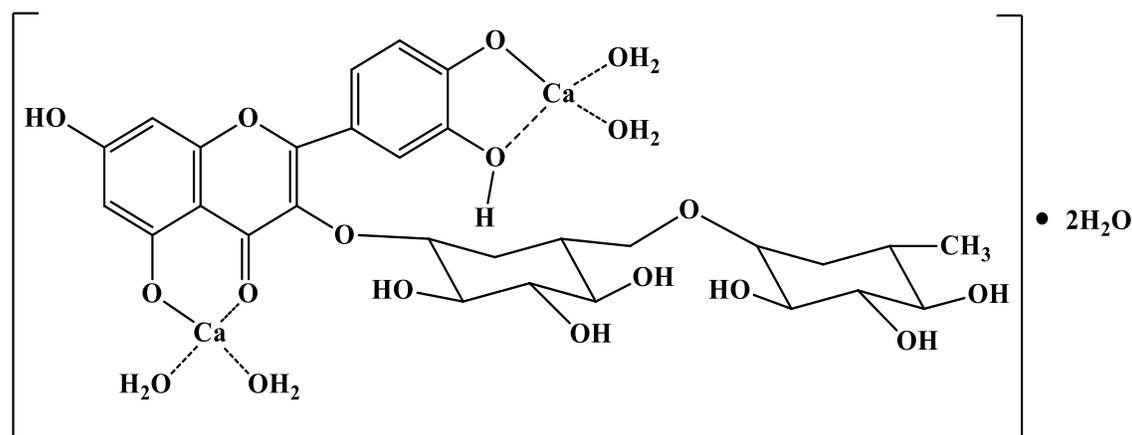


Fig.8. Proposed structure of the Ca (II)-rutin complex

3.6 The level of blood-lipid in rats

The serum total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C) and triglyceride (TG) levels of the model group were significantly different from those of the normal control group and the experimental group ($P < 0.05$). But the serum high-density lipoprotein cholesterol (HDL-C) level of the model group was not significantly different from those of the normal control group. The serum TC, LDL-C and TG levels of the experimental group were higher than those of the normal control group but lower than those of the model group, and the serum HDL-C level was significantly higher than those of the normal control group and the model group ($P < 0.05$). The main data are summarized in table 3.

3.7 The viscera index of the rats

The main data are summarized in table 4. The liver and spleen index of the model group were significantly higher than those of the normal group and the experimental group ($P < 0.05$), while no significant difference was shown between the normal group and the experimental group. There was no significant difference between cardiac index and renal index.

The liver index of the rats was positively correlated with triglyceride ($r = 0.453$, $P < 0.05$), total cholesterol ($r = 0.491$, $P < 0.01$) and low-density lipoprotein cholesterol ($r = 0.533$, $P < 0.01$), but not obviously correlated with high-density lipoprotein cholesterol ($r = 0.388$, $P < 0.05$). The spleen index of the rats was correlated with TG ($r = 0.388$, $P < 0.05$), but not obviously correlated with TC, HDL-C and LDL-C. The cardiac index and renal index were not obviously correlated with the serum lipid level.

It indicated that the hyperlipidemia induced by lipid emulsions might cause the abnormal enlargement of the liver and spleen, which was similar to the clinical symptom of hyperlipidemia. However, the rutin

calcium complex could improve the various indexes of serum lipid and decrease the spleen index and liver index.

4 Conclusion

There were two ways to prepare the experimental hyperlipemia model. One was to feed the animals with high lipid diet. The other was that the animals were administrated by gastric perfusion of fat emulsion. Of the two, the latter was better than the former, because the latter could assure a coherent condition, and we could regulate the content of cholesterol, lard according to the actual condition. Also the time of model preparation was short. In this experiment, the hyperlipemia model was established successfully by gastric perfusion of fat emulsion.

In this study, disorder of lipometabolism was detected in the model group rats. Compared with those of the normal control group, the serum TC, TG levels of the model group were much higher, and it caused hyperlipidemia rats. But the serum TC, TG and HDL levels of the experimental group indicated that rutin-calcium complex was quite effective in reducing serum TC and TG and adjusting blood lipid.

As a part of the research project, a new complex of Ca (II) with rutin was prepared and characterized. This study examined the interaction of metal ion with rutin in methanolic solution. The spectroscopic data showed the importance of 5-OH group as a coordination site. The complex had been characterized on the basis of analytical and spectral data. The results showed that rutin-calcium complex had the effect of anti-hyperlipemia and protecting pathological changes of organs in rats with experimental hyperlipemia.

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