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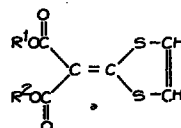


(54) PHARMACEUTICAL COMPOSITION FOR CURING LIVER DISEASES

(71) We, NIHON NOHYAKU CO. LTD., a corporation organised under the laws of Japan, of 1-2-5, Nihonbashi, Chuo-ku, Tokyo, Japan, do hereby declare this invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

This invention relates to a pharmaceutical composition for controlling the liver diseases of humans and animals.

More particularly, the invention is concerned with a pharmaceutical composition for preventing, alleviating and curing liver diseases containing an effective amount of a compound having the general formula (I),



(I)

wherein R¹ and R² which may be same or different, represent individually a C₁—C₄ alkyl group, and a pharmaceutically acceptable diluent.

The invention further relates to a pharmaceutical composition for lowering the concentration of alcohol in the blood and abnormally elevated concentration of sugar in the blood, which contains a compound of the above-mentioned general formula (I).

The invention further pertains to the use of the said compound as a liver diseases controlling agent and a blood alcohol- or abnormally elevated blood sugar-lowering agents.

In view of its various functions, the liver is frequently called a delicate chemical factory. Thus, in the liver, various chemical reactions are being biochemically effected, such as detoxication, sugar metabolism, protein metabolism, lipid metabolism, formation and secretion of bile, control of hormones, formation of blood coagulant prothrombin, regeneration of liver cells, and storage of various living body-constituting elements (fats, glycogens, proteins and vitamins).

However, even such delicate and well-balanced functions of the liver sometime undergo disorders, either acutely or chronically, due to various factors such as alcohol, insufficient nutrition, viruses, drugs, toxicants, damage of bile flow, lesions of blood and lymphatic vessels in the liver, etc. to cause such diseases as, for example, fatty liver, toxic liver damages, alcoholic hepatitis, viral hepatitis, congestion of the liver, cholestasis, jaundice, and cirrhosis or hepatoma as a final stage.

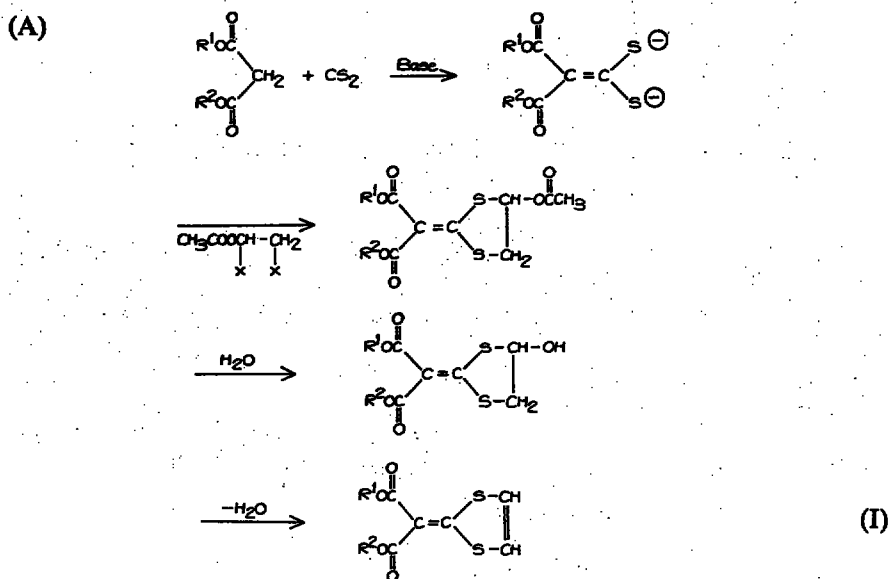
As the result of extensive studies, the present inventors have found that compounds represented by the aforesaid general formula (I) have actions to activate liver cells and to activate liver cells and metabolic functions of the liver, such as sugar metabolism, detoxication (for example, detoxication of alcohol),

formation and excretion of bile and biliary salts (chloretic action) and regeneration of the liver.

The inventors have also found that the compounds represented by general formula (I) have a pharmacological activity to alleviate or cure the existing liver diseases.

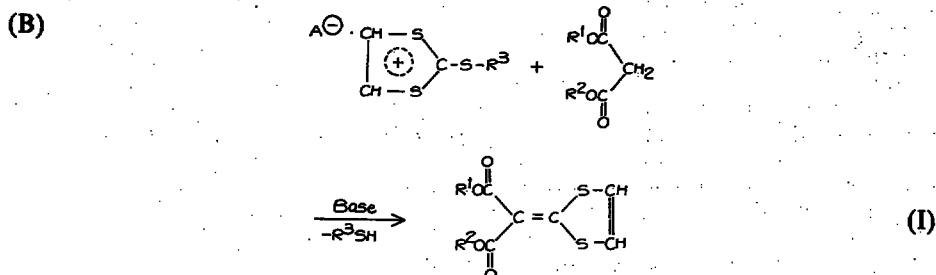
The inventors have further found that the compounds represented by general formula (I) have a pharmacological activity to protect the function of the liver against a certain kind of damage or load.

The compounds represented by the aforesaid general formula (I) are novel compounds, and can be synthesized, in practice, according to any of the two typical synthesis processes shown below by way of the reaction schema (A) and (B).



wherein R^1 and R^2 are as defined previously; and X represents a halogen atom.

That is, according to this process, the compounds of the general formula (I) can be synthesized by reacting a malonic acid ester with carbon disulfide in the presence of a suitable base to form a dithiolate salt, reacting the dithiolate salt with a 1-acetoxy-1,2-dihalogenoethane to prepare a dialkyl 4-acetoxy-1,3-dithiolan-2-ylidene malonate, hydrolyzing the thus prepared malonate under proper conditions, and then subjecting the resulting dialkyl 4-hydroxy-1,3-dithiolan-2-ylidene malonate to dehydration reaction under proper conditions. The dehydration reaction is advantageously carried out in the presence of a dehydrating agent. Examples of the dehydrating agent include concentrated sulfuric acid, chlorosulfonic acid, methanesulfonic acid chloride, benzenesulfonic acid or its chloride, p-toluenesulfonic acid, phosphorus oxychloride, phosphorus pentachloride and phosphorus pentoxide, though these are not limitative. Further, examples of the inert solvent used in the dehydration reaction include carbon tetrachloride, benzene, and ethers, though these are not limitative.

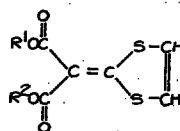


wherein R^1 and R^2 are as defined previously; A^- represents an anion residue; and R^3 represents an alkylating agent residue.

That is, according to this process, the compounds of the general formula (I) can be synthesized by reacting a dithiolium salt, which can be obtained by the reaction of 1,3-dithiol-2-thion with an alkylating agent or by the decarboxylation and simultaneous alkylation of a 1,3-dithion-2-thion-4,5-dicarboxylic acid, with a malonic acid ester in the presence of a suitable base. Examples of the solvent used in the above reaction are alcohols, acetic acid, dimethylformamide, dimethyl sulfoxide and tetrahydrofuran, though these are not limitative. Preferable as the base are alcoholates, sodium hydroxide, potassium hydroxide and sodium hydride, though these are not limitative.

Typical examples of the compounds represented by the general formula (I) are as shown in Table 1.

TABLE 1



Compound No.	R ¹	R ²	m.p. or refractive index
1	CH ₃	CH ₃	m.p. 134.5° – 135°C
2	C ₂ H ₅	C ₂ H ₅	m.p. 113°C
3	n-C ₃ H ₇	n-C ₃ H ₇	m.p. 73° – 75°C
4	i-C ₃ H ₇	i-C ₃ H ₇	m.p. 59° – 60°C
5	n-C ₄ H ₉	n-C ₄ H ₉	m.p. 55° – 57°C
6	i-C ₄ H ₉	i-C ₄ H ₉	m.p. 76° – 78°C
7	C ₂ H ₅	i-C ₃ H ₇	m.p. 57° – 58°C
8	CH ₃	i-C ₄ H ₉	n _D ²⁰ 1.5928

The compounds represented by the general formula (I) are extremely low in toxicity to mammals, and their acute oral toxicity to male mice expressed as LD₅₀ values are at such a low toxicity level as in the range from 1,000 to 6,000 mg/kg of body weight or more, in general. For example, the LD₅₀ value of the compound 4 is 3,120 mg/kg. Further, these compounds have no detrimental effects on test animals administered therewith, so far as the doses thereof are within an ordinary administration range.

There are known a variety of causal factors including toxic liver damage, hepatitis and fatty liver. The predominant changes observed in these diseases are necrosis of liver cells, mesenchymal reaction and accumulation of lipid.

The feature of necrosis depends on the causal factor. It can be classified into centrilobular necrosis, periportal necrosis and discrete lobular necrosis.

These changes are known to be realized experimentally on model by administering the following chemicals to laboratory animals.

Centrilobular necrosis is induced with carbon tetrachloride, thioacetamide, chloroform or bromobenzene.

Periportal necrosis can be induced with allyl alcohol.

Discrete lobular necrosis associated with mesenchymal reaction can be induced with D-galactosamine.

Fatty liver can be induced on model by administering carbon tetrachloride or ethionine.

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All these acute, subacute and chronic liver damages are known to transform into cirrhosis as the final stage. In laboratory, a model of cirrhosis can be induced on model by a longterm repeated administration of carbon tetrachloride to animals.

Experiments have revealed that the compounds represented by the aforesaid general formula (I) have such broad and various pharmacological activities as mentioned below:

- (1) Activities to cure (including to prevent and to alleviate, hereinafter the same) liver diseases associated with centrilobular necrosis;
- (2) Activities to cure liver diseases associated with periportal necrosis;
- (3) Activities to cure liver diseases associated with discrete lobular necrosis and mesenchymal reaction;
- (4) Activities to cure fatty liver;
- (5) Activities to cure cirrhosis;
- (6) Activities to cure toxic liver damages;
- (7) Activities to cure congestion of the liver;
- (8) Activities to stimulate the excretion of bile and biliary salts (choleretic activity);
- (9) Activities to lower the concentration of alcohol in the blood;
- (10) Activities to lower abnormally elevated concentration of sugar in the blood; and
- (11) Activities to alleviate the toxicity of metallic salts such as selenium salts and cadmium salts.

As above, the compounds represented by general formula (I) have a pharmacological activity to alleviate or cure the existing liver disease and to protect the liver against a certain kind of damage or load. Accordingly, the compounds are useful as pharmaceutical for controlling the acute or chronic liver diseases of humans and animals induced by various causes. According to this invention, the compounds represented by general formula (I) can be used as pharmaceutical for therapying fatty liver, alcoholic hepatitis, viral hepatitis, toxic liver damages, congestion of the liver, cholestasis, and cirrhosis as the final stage.

On the other hand, histopathological observation has revealed that the compounds represented by general formula (I) have activities to cure centrilobular necrosis, periportal necrosis and discrete lobular necrosis associated with mesenchymal reaction found in the liver. Accordingly, they are useful as pharmaceutical for controlling necrotic liver diseases of humans and animals.

The compounds represented by general formula (I) have an action to activate liver cell and metabolic function of the liver, including the excretion of bile and biliary salt, the sugar metabolism and the detoxication of hepatotoxins such as alcohol. Accordingly, they are useful as cholagogic or curative for jaundice of humans and animals.

The compounds represented by general formula (I) are also useful as pharmaceutical for lowering abnormally elevated sugar concentration in the blood. Accordingly, they can be used as a diabetes curative for humans and animals. This usage also belongs to the scope of this invention.

The compounds represented by general formula (I) are also useful as pharmaceutical for lowering alcohol concentration in the human blood or as an antidote. Accordingly, they can be used as pharmaceutical for therapying alcoholic temulence or hang-over. This usage also belongs to the scope of this invention.

The terms "pharmaceutical for controlling liver diseases" referred to in this invention mean a pharmaceutical of which efficacy is based on the various pharmacological activities manifested in the liver by the compounds represented by general formula (I), such as the abovementioned activities (1) to (11). The scope of object to which the pharmaceutical of this invention can be administered involves humans and animals.

In using the compounds represented by general formula (I) as pharmaceutical for controlling liver diseases, they are used in the form suitable for exhibiting the intended efficacy. That is, they can be used as pharmaceutical for controlling liver diseases in themselves alone. Otherwise, they may also be formulated, according to usual procedures and means adopted in this field, into pharmaceutical compositions in the form of a mixture with a pharmaceutically acceptable diluent and/or other pharmacologically active substance. Otherwise, it is also permitted to provide the pharmaceutical of this invention in the form of administration units convenient for their individual application purposes which contain the compound.

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of this invention alone or contain it in mixture with a pharmaceutically acceptable diluent and/or other pharmacologically active substance.

The pharmaceutical of this invention can be administered either orally or parenterally. Accordingly, the pharmaceutical of this invention can take an appropriate shape suitable for oral or parenteral administration.

It is also permitted to provide the pharmaceutical of this invention in the form of administration units. In this case, the pharmaceutical contains an effective amount of active ingredient compound. Said effective amount should mean a certain range in accordance with the changes in various conditions.

The shape in which the pharmaceutical of this invention can be provided includes, for example, powders, granules, tablets, sugar-coated tablets, pills, capsules, suppositories, suspensions, liquids, emulsions, ampoules, injections and the like.

The pharmaceutical of this invention is provided, in accordance with needs, in the aseptic state or in the form of an isotonic aqueous solution.

This invention includes such mode that the compound of general formula (I) is administered in the form of a mixture with a pharmaceutically acceptable diluent. The diluent referred to herein means a material other than the compounds represented by general formula (I). The diluent may be a solid, a semisolid, a liquid or an ingestible capsule. Examples of the diluent include vehicles, extenders, binders, wetting agents, disintegrators, surfactants, lubricants, dispersants, buffer agents, seasonings, deodorants, dyes, flavors, preservatives, dissolution aids, solvents and the like, though these are not limitative. These diluents may be used either singly or in the form of a mixture of two or more members. Such pharmaceutically acceptable diluents as above are sometimes used in the form of a mixture with other pharmacologically active substance, which also belongs to the scope of this invention.

The pharmaceutical composition of this invention may be prepared according to any known method. For example, a mixture of the active ingredient and a diluent is formed, for example, into granules, and thus formed granular composition is molded, for example, into tablets. In case the pharmaceutical composition is for parenteral administration, it is preferable to be made aseptic and, if necessary, be made isotonic to the blood.

Generally, the pharmaceutical composition of this invention contains at least 0.01% by weight, based on the weight of the composition, of the active compound. Thus, this invention includes such mode that the said compound is used independently.

In case the compound of this invention is formulated into administration units, individual constituents forming the formulated composition may be either same or different in the shape. Most frequently adopted shapes include, for example, the followings: tablets, granules, pills, powders, sugar-coated tablets, capsules, ampoules and the like.

For the control of various liver damages and various diseases derived therefrom, the pharmaceutical composition of the present invention may be applied to humans and animals according to an ordinary procedure adopted in this field, in order to attain such effects as shown in the aforesaid animal tests. Thus, the composition of the present invention is administered orally or parenterally. The oral administration includes sublingual administration, and the parenteral administration includes administration by way of injection including, for example, subcutaneous, intramuscular and intravenous injections and instillation.

The dose of the pharmaceutical of this invention varies depending on many factors, including the kind of subject (whether the pharmaceutical is administered to humans or to animals, the difference in susceptibility, age, sex, body weight, the clinical picture, the physical conditions of patients, the means of administration, the time and interval of administration, the kind and properties of pharmaceutical composition, the kind of active ingredient, etc. In some cases, accordingly, the dose of the pharmaceutical may be made smaller than the minimum dose mentioned below, while in other cases the dose would be in excess of the maximum dose mentioned below. In case the pharmaceutical is to be administered in a large dose, it is preferable that the pharmaceutical is divisionally administered several times a day.

In the case of oral administration, effective dose for animals is in the range from 0.1 to 500 mg, preferably from 1 to 100 mg, of active ingredient per one kilogramme body weight per day. In the case of parenteral administration, effective dose for animals is in the range from 0.01 to 250 mg, preferably from 0.1

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to 25 mg, of active ingredient per one kilogramme body weight per day.

In the case of oral administration, effective dose for humans, deduced from the above-mentioned effective dose for animals with consideration for susceptibility difference and security, is advantageously in the range from 0.1 to 250 mg, preferably from 0.5 to 50 mg, per one kilogramme body weight per day. In the case of parenteral administration, effective dose for humans is in the range from 0.01 to 100 mg, preferably from 0.1 to 25 mg, per one kilogramme body weight per day.

The present invention is illustrated in more detail below with reference to examples including synthesis examples, but the invention is not limited to the examples.

Synthesis Example 1.

Synthesis of diisopropyl 1,3-dithiol-2-ylidene malonate.

(Compound 4 in Table 1)

To a solution of 3 g of diisopropyl 4-hydroxy-1,3-dithiolan-2-ylidene malonate in 50 ml of carbon tetrachloride was added 1.2 g of chlorosulfonic acid, and the resulting mixture was continuously stirred at room temperature for 2 hours. After completion of the reaction, water was added to the reaction liquid, and the carbon tetrachloride layer was separated. The aqueous layer was extracted with ether, and then combined with the carbon tetrachloride layer. Subsequently, the combined layer was dried, and then the solvent was removed by distillation to form crystals. The thus formed crystals were collected and then dried to obtain 2.5 g of the abovementioned compound in the form of white crystals, m.p. 59°—60°C, yield 86%.

Synthesis Example 2.

Synthesis of diisopropyl 1,3-dithiol-2-ylidene malonate.

(Compound 4 in Table 1)

A solution of 44.4 g (0.2 mole) of 1,3-dithiol-2-thion-4,5-dicarboxylic acid in 240 ml of nitromethane was heated to 80°C. Into this solution, 100 ml of methyl iodide was gradually dropped, and the resulting mixture was refluxed for 6 hours. After completion of the reaction, the formed crystals were recovered by filtration, washed with 100 ml of ether and then air-dried to obtain 48.4 g of 2-methylthio-1,3-dithiolium iodide, m.p. 114°—116°C (decomp.).

Separately, into a suspension of 1.1 g (0.03 mole) of 69% purity sodium hydride in 30 ml of dry tetrahydrofuran was gradually dropped with ice-cooling 5.6 g (0.03 mole) of diisopropyl malonate. After the generation of hydrogen gas from the resulting mixture had terminated, 8.2 g (0.03 mole) of the previously obtained 2-methylthio-1,3-dithiolium iodide was added to the mixture. Subsequently, the mixture was refluxed for 1 hour, and then the reaction liquid was poured into a large amount of ice water to deposit crystals. The crystals were recovered by filtration, dried, and then recrystallized from n-hexane to obtain 6.7 g of the abovementioned compound in the form of white crystals, m.p. 59°—60°C, yield 77.5%.

In Examples 1 to 7 described below, all parts are by weight.

Example 1.

Diethyl 1,3-dithiol-2-ylidene malonate (Compound 2)	10 parts
Heavy magnesium oxide	10 "
Lactose	80 "

The above-mentioned components were homogeneously mixed and pulverized to obtain a powder.

Example 2.

Di-n-propyl 1,3-dithiol-2-ylidene malonate (Compound 3)	10 parts
Synthetic aluminum silicate	10 "
Calcium hydrogenphosphate	5 "
Lactose	75 "

The above-mentioned components were treated in the same manner as in Example 1 to obtain a powder.

Example 3.

5	Diisopropyl 1,3-dithiol-2-ylidene malonate (Compound 4)	50 parts	5
	Starch	10 „	
	Lactose	15 „	
	Crystalline cellulose	20 „	
	Polyvinyl alcohol	5 „	
10	Water	30 „	10

The above-mentioned components were homogeneously kneaded, granulated, dried and sieved to obtain a granule.

Example 4.

15	99 Parts of the granule obtained in Example 3 was incorporated with 1 part of calcium stearate, and then subjected to compression molding to obtain a tablet of 10 mm in diameter.	15
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Example 5.

	Ethyl isopropyl 1,3-dithiol-2-ylidene malonate (Compound 7)	95 parts	
20	Polyvinyl alcohol	5 „	20
	Water	30 „	

25	The above-mentioned components were treated in the same manner as in Example 3 to obtain a granule. 90 Parts of the thus obtained granule was incorporated with 10 parts of crystalline cellulose, and then subjected to compression molding to obtain a tablet of 8 mm in diameter. Further, this tablet was formed into a sugar-coated tablet by use of proper amounts of a suspension comprising ethanolic shellac, syrup, gelatin and precipitated calcium carbonate and a dye.	25
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Example 6.

30	Diisopropyl 1,3-dithiol-2-ylidene malonate (Compound 4)	0.5 part	30
	Nonionic surfactant	2.5 parts	
	Isotonic sodium chloride solution	97.0 „	

35	The above-mentioned components were mixed together with heating to form a solution, which was then cooled to obtain an injection.	35
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Example 7.

The powder obtained in Example 1 was filled into commercially available capsules to obtain a capsule.

Example 8.

40	Protective Effect on Acute Liver Damage Associated with Centrilobular Necrosis. (Experimental Model using Carbon Tetrachloride) Carbon tetrachloride (CCl ₄) administration induces centrilobular necrosis of the liver associated with loss of diphosphopyridine nucleotide, hepatic glycogen, coenzyme A and increase in neutral fat. Release of several enzymes from the	40
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hepatocytes, and increase of enzyme activities in the plasma are recognized as the result of the damage of the liver. A suitable means for evaluating the degree of damage induced by CCl_4 or the degree of protection afforded by drugs is to study the plasma glutamic-pyruvic transaminase (p-GPT) activity.

5 *Methods*

10 The test compounds were dissolved or suspended in olive oil and administered orally at the dose of 250 mg/kg to the mice (four-week-old male mouse-dd strain). After 6 hours, CCl_4 was administered orally (0.05 ml/kg as olive oil solution). Animals were killed 24 hours after CCl_4 administration, and the liver was grossly observed. The plasma was obtained by centrifugation. Activities of p-GPT were determined by the method of Reitman and Frankels and expressed in Karmen units.

Score for liver damage index was as follows:

	Liver damage index	Description
15	0	Normal
	2	Slightly recognised
	4	Clearly observable damage
	6	Heavy damage

20 Each figure indicates average of 5 to 6 mice. Values of p-GPT over 1,000 Karmen unit regarded as 1,000 for calculation of average for convenience.

Results

TABLE 2.

Protective effect on acute liver damage associated with centrilobular necrosis.

Compound No.	Liver Damage Index	p-GPT
1	0.4	138
2	0.2	36
3	0.1	36
4	0.0	36
5	0.4	52
6	0.4	75
7	0.8	102
8	0.6	88
Carbon tetrachloride alone	5.2	>1,000
Thioctic acid amide	4.8	763
Anethol trithion	1.6	38
Control	0.0	36

Carbon tetrachloride is well known to induce centrilobular necrosis in the liver. As is clear from the results of tests carried out by use of carbon tetrachloride, all the compounds in the invention showed prominent protective effect on acute liver damage, and are comparable in effectiveness to thioctic acid amide and anethol trithion which are commercially available at present as drugs for liver diseases.

As above, the compound of this invention is useful as a pharmaceutical for use in controlling the human and animal liver diseases associated with centrilobular necrosis.

The dose of test compound varies depending on the degree of the liver damage. When the liver damage in the model is not serious, the dose of test compound can be reduced.

For instance, the compound of this invention is found to be effective even at a dose as small as 1 to 2 mg/kg when the quantity of carbon tetrachloride administered to animal is 0.005 ml/kg.

Example 9.

Protective Effect on Acute Liver Damage Associated with Centrilobular Necrosis.
(Experimental Models using Bromobenzene, Dimethylnitrosamine and Chloroform)

Bromobenzene (hereinafter abbreviated as BB), Dimethylnitrosamine (hereinafter abbreviated as DMN) and chloroform (hereinafter abbreviated as CF) resemble carbon tetrachloride in inducing liver damage associated with centrilobular necrosis.

Testing Method

Test compound or anethol trithion was orally administered to animal at the dose of 100 mg/kg. Six hours after it, the hepatotoxin was administered at such a dose and via such a route as shown in Table 3. Twenty four hours after it (in the case of dimethylnitrosamine, exceptionally fifteen hours after it) the animals were sacrificed and blood samples were collected. Indices of hepatotoxicity adoptable in each chemical were measured, from which the efficacy of the compound was evaluated.

TABLE 3.

Administration of hepatotoxin and index of hepatotoxicity

Hepatotoxin	Animal	Dose (ml/kg)	Route	Index of hepatotoxicity
BB	male rat (SD)	0.75	oral	p-GPT activity BSP test
DMN	male mouse (4 week-old, dd)	0.05	intraperitoneal	p-GPT activity BSP test
CF	male mouse (4 week-old dd)	0.2	oral	p-GPT activity

Results

Similarly to carbon tetrachloride, BB, DMN and CF are known to be hepatotoxins inducing centrilobular necrosis. As shown in Table 4, Compound 4 of this invention normalized the indices of hepatotoxicity to demonstrate its high efficacy for preventing liver damage.

In addition, it was superior to anethol trithion in the effect.

Thus, it is evident that the compound of this invention is useful as a pharmaceutical for controlling human and animal liver diseases accompanied with centrilobular necrosis.

TABLE 4.

Protective effects on acute liver damages associated with centrilobular necrosis

Hepatotoxin	Index of hepatotoxicity	Control	Positive control*	Compound 4 **	Anethol trithion **
BB	p-GPT (Karmen unit)	16 $\pm$ 1.9	53 $\pm$ 27	23 $\pm$ 3.7	28 $\pm$ 4.1
	BSP (mg/dl)	2.7 $\pm$ 1.7	20.4 $\pm$ 7.1	10.6 $\pm$ 4.5	12.9 $\pm$ 5.5
DMN	p-GPT	15 $\pm$ 1.5	>600	204 $\pm$ 44	192 $\pm$ 54
	BSP	1.5 $\pm$ 0.3	31.2 $\pm$ 5.9	10 $\pm$ 2.0	17.6 $\pm$ 2.7
CF	p-GPT	20 $\pm$ 3.0	432 $\pm$ 58	58 $\pm$ 15	>1000

*: Hepatotoxin alone was administered.

**: After treatment with Compound 4 or anethol trithion, the hepatotoxin was administered.

Example 10.

Therapeutic Effect on Chronic Liver Damage.
(Experimental Model using Thioacetamide)

Thioacetamide (hereinafter abbreviated as "TAA") also causes liver damages, like carbon tetrachloride, and hence is frequently used as a chemical for bringing liver damage and fatty liver diseases. In the tests of this Example, TAA was repeatedly administered to animals to induce chronic liver damages, and then the present compound was administered thereto to know whether or not the compound was effective against chronic liver damage.

Methods

Five groups of the male rats (Sprague Dawley strain) were treated as follows:

Group A: The rats were orally administered with 100 mg/kg of thioacetamide, at 3 days intervals for 36 days (12 times of the administration), then were submitted for 10 days to a normal diet.

Group B: The rats were orally administered with 100 mg/kg of thioacetamide at 3 days intervals for 36 days (12 times of the administration), then were submitted for 10 days to the normal diet + 0.2% of compound 4.

Group C: The rats were submitted to the normal diet as the control.

Five rats were sacrificed from each group at appropriate intervals for BSP (sulfobromophthalein) test, the results of which were shown as amounts (mg) of BSP remaining in 1 dl of plasma.

ResultsTABLE 5.
Therapeutic effect on chronic liver damage

Time of Sacrifice	Group A	Group B	Group C
24 hr. after 4 times TAA administration	18.2 ± 2.6		0.3 ± 0.1
24 hr. after 8 times TAA administration	16.8 ± 5.2		—
24 hr. after 12 times TAA administration	17.3 ± 4.4		0.8 ± 0.1
After compounds administration			
2 Days	12.7 ± 3.7	7.2 ± 2.5	0.4 ± 0.1
5 Days	8.7 ± 1.9	2.8 ± 0.7	—
10 Days	1.4 ± 0.4	0.5 ± 0.1	0.6 ± 0.1

By the repeated administration of TAA, the concentration of BSP in the blood increased to 16 to 19 mg/dl and the said level lasted, and therefore it is considered that the rats were brought to a state close to chronic liver damage. After the administration of TAA, the present compound-administered group (Group B) was quicker in cure of liver damage than the unadministered group (Group A).

This indicates that the present compound is effective against subacute or chronic liver damage or hepatitis in humans and animals.

Example 11.

Protective Effect on Acute Liver Damage Associated with Periportal Necrosis.
(Experimental Model using Allyl Alcohol)

Testing method

The compound of this invention or anethol trithion was orally administered to the male mouse (4 week-old, dd strain) at the dose of 100 mg/kg. Six hours after it, allyl alcohol was orally administered at the dose of 0.075 ml/kg. Twenty four hours after it, the animals were sacrificed to collect blood samples. BSP test revealed the residual quantity of BSP, from which the protective effect of the compound of this invention was evaluated.

ResultsTABLE 6.
Protective effect on acute hepatitis

Hepatotoxin	Index of hepatotoxicity	Control	Positive control *	Compound 4**	Anethol trithion **
Allyl alcohol	BSP	1.8 ± 0.4	28.4 ± 4.8	17 ± 3.2	19.3 ± 4.6

*: Hepatotoxin alone was administered.

**: After treatment with Compound 4 or anethol trithion, the hepatotoxin was administered.

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Allyl alcohol differs from carbon tetrachloride or bromobenzene, dimethylnitrosamine or chloroform in that it induces liver damage associated with periportal necrosis. As shown in Table 6, the compound of this invention protected the liver damage.

Accordingly, the compound of this invention is useful as a pharmaceutical for human or animal liver disease accompanied with periportal necrosis.

Example 12.

Protective Effect on Acute Hepatitis associated with Mesenchymal Reaction and Discrete Lobular Necrosis.

(Experimental Mode using D-Galactosamine)

D-Galactosamine is a compound which induces a discrete lobular necrosis associated with mesenchymal reaction similar to the change observed in human viral hepatitis, so that it is frequently used in producing a model for viral hepatitis.

Methods

The compound of this invention or anethol trithion was administered orally to the male rate (SD strain) at the dose of 100 mg/kg. Six hours after it, D-galactosamine was intraperitoneally administered at the dose of 600 mg/kg. Four hours after it, an additional 300 mg/kg was administered intraperitoneally. Eight hours after it, the animals were sacrificed to collect the blood samples. GPT activity and triglyceride in the liver were measured to evaluate the effect of the compound of this invention.

Results

TABLE 7.

Protective effect on acute liver damage associated with discrete lobular necrosis.

Hepatotoxin	Index of hepatotoxicity	Control	Positive control *	Compound 4**	Anethol trithion **
D-Galactosamine	GPT (Karmen unit)	13 ± 0.5	92 ± 59	51 ± 15	106 ± 30
	Triglyceride in the liver (mg/g)	6.5 ± 1.2	8.2 ± 2.2	4.4 ± 0.68	9.2 ± 1.5

*: Hepatotoxin alone was administered.

**: After treatment with Compound 4 or anethol trithion, the hepatotoxin was administered.

As shown in Table 7, the compound of this invention protected the liver damage.

Accordingly, the compound of this invention is useful as a pharmaceutical for use in therapying human or animal hepatitis accompanied with mesenchymal reaction and discrete lobular necrosis.

Example 13.

Effect on Fatty Liver.

(Experimental Model using Ethionine)

There are known many factors inducing fatty liver. But, the fatty liver is grouped into a couple of patterns from the mechanism of lipid accumulation or metabolism of lipoprotein in the liver. Ethionine is a typical example which induces first pattern of fatty liver. It inhibits RNA and protein synthesis and destructs polysome of the liver cell. Fatty liver is induced by inhibition of the protein synthesis and disturbance of lipoprotein secretion.

Usually, fatty liver is closely related to the accumulation of triglyceride in the liver. In the present example, the degree of fatty liver and protective or therapeutic effect on the fatty liver was evaluated by measurement of triglyceride content in the liver.

Methods

The compounds of this invention and methionine were independently dissolved or suspended into olive oil and administered orally to the male mouse (4 week-old dd-strain) at the dose of 250 mg/kg. One hour before it, simultaneously with it or one hour after it, ethionine dissolved in equimolar NaOH solution was administered intraperitoneally at the dose of 200 mg/kg. Twenty four hours after the administration of ethionine, the animals were sacrificed and triglyceride content in the liver was determined by chromotropic acid method.

Results

TABLE 8.

Effect on fatty liver

Group	Number of animals	Triglyceride (mg/g-liver)
Control	14	4.0 ± 0.33
Ethionine	14	13.6 ± 1.08
Compound 4 → 1 hr. → Ethionine	14	9.1 ± 1.15
Compound 4 → 0 hr. → Ethionine	15	5.4 ± 0.61
Ethionine → 1 hr. → Compound 4	15	10.0 ± 1.15
Methionine → 0 hr. → Ethionine	15	10.3 ± 0.83

As above, the compounds of this invention depress the abnormal accumulation of triglyceride, induced by ethionine, in the liver to exhibit a protective and therapeutic effect against fatty liver. The compounds of this invention are superior to methionine in the above-mentioned effect. Accordingly, the compounds of this invention are useful as pharmaceutical for human and animal fatty liver.

Example 14.

Therapeutic Effect against Fatty Liver.
(Experimental Model using Carbon Tetrachloride)

Carbon tetrachloride also induces fatty liver, but the picture of disease differs from the case of ethionine-induced fatty liver. Carbon tetrachloride is generally considered to damage microsome and thereby to inhibit protein synthesis and induce fatty liver.

Methods

Carbon tetrachloride was subcutaneously administered to 35 week-old male rats (SD strain) for 4 days at the dose of 1 ml/kg. The treated animals were left for 3 days after the last administration to maximize the manifestation of fatty liver.

Administration of Compound 4 was commenced on the third day after the administration of carbon tetrachloride was completed. It was orally given every day for 10 days at the dose of 50 or 250 mg/kg. On the 11th day, the animals were sacrificed by exsanguination. The therapeutic effect was evaluated by determining the content of lipid in the liver (triglyceride and total lipid) and examining the histopathological changes. Triglyceride and total lipid were determined

colorimetrically by chromotropic acid method and Brangdon's oxidation method, respectively.

Indices for the histopathological change is as follows:

Index	Histopathological changes
-	Normal
±	Formation of small droplet deposition of lipid but the number of droplet is not so many.
+	Formation of small or slightly fused droplet deposition of lipid and increasing the number of droplet.
++	Formation of fused large droplet deposition of lipid.

Results

TABLE 9.

Therapeutic effect on fatty liver

Group	Triglyceride (mg/g-liver)	Total lipid (mg/g-liver)
Control	13.0 ± 3.6	64.3 ± 8.3
Positive control	50.9 ± 8.9	151.3 ± 24.0
Compound 4 (50 mg/kg)	37.6 ± 8.5	78.6 ± 16.8
ibid (250 mg/kg)	23.6 ± 7.0	64.1 ± 10.2
Methionine (250 mg/kg)	47.3 ± 7.3	146.1 ± 13.0

TABLE 10

Therapeutic effect on fatty liver (Histopathological examination)

Group	Degree of fatty liver							
Control	-	-	-	-	-	-	-	-
Positive control	+	+	+	+	++			
Compound 4 (50 mg/kg)	+	±	±	±	±	-	±	
ibid. (250 mg/kg)	-	-	±	±	±	±	-	
Methionine (250 mg/kg)	+	±	++	+	++	+	+	

As shown in Table 9, the group "Positive control" to which carbon tetrachloride was administered but thereafter the compound of this invention was not administered manifested a high degree of fatty liver and showed no sign of improvement.

On the other hand, in the therapeutic groups to which the compound of this invention was given at the dose of 50 or 250 mg/kg the degree of fatty liver was significantly improved. In other words, the compound of this invention exhibited a therapeutic effect.

On the contrary, methionine hardly exhibited a therapeutic effect against fatty liver induced by carbon tetrachloride.

Table 10 illustrates the results of histopathological examination. The size and number of lipid droplet decreased in the groups therapied with the compounds of this invention, demonstrating an alleviation of fatty liver.

Accordingly, the compound of this invention is useful as pharmaceutical for fatty liver in humans and animals.

Example 15.

Therapeutic Effect on Cirrhosis.

Almost all the liver diseases induced by various causes are considered to advance, finally, to cirrhosis. The cirrhosis is a final stage of liver damage, which is difficult to classify from the causal viewpoint. Usually, experimental cirrhosis is induced by longterm repeated administration of carbon tetrachloride. The therapeutic effect of the compounds of this invention on cirrhosis, which had been caused by carbon tetrachloride administration, was examined.

Methods

A 10% solution of carbon tetrachloride in olive oil was administered to the male rat (4 week-old SD-strain) intraperitoneally at the dose of 0.5 ml carbon tetrachloride/kg twice a week over a period of 14 weeks, to induce cirrhosis. Four days after the final administration of carbon tetrachloride, the formation of pseudo lobulus was confirmed. Then the diseased animals were divided into two groups. Therapeutic group was submitted a diet containing 2000 ppm of Compound 4, while the positive control group was submitted a normal diet. Control group, to which olive oil alone had been administered, was submitted a normal diet.

At 1, 2, 4 and 8 weeks after beginning of Compound 4 feeding, 5 animals from each group were sacrificed, and the therapeutic effect on cirrhosis was evaluated by examining plasma transaminase activity (p-GPT and p-GOT, Reitman-Frankel method) and observing histopathological changes.

Degree of cirrhosis in the histopathological examination was graded as follows:

Index	Histopathological changes
-	Normal
±	Slight formation of pseudo lobulus
+	Slight formation of pseudo lobulus, and slight fibrosis
++	Moderate formation and cohesion of pseudo lobulus, moderate fibrosis
+++	Severe cohesion of pseudo lobulus, severe fibrosis

Results

TABLE 11.

Therapeutic effect on cirrhosis (Transaminase activity)

Period of therapy (week)	p-GPT activity (Karmen unit)			p-GOT activity (Karmen unit)		
	Control	Positive control	Therapeutic group	Control	Positive control	Therapeutic group
0	28 ± 3	262 ± 46		79 ± 6	658 ± 127	
1	33 ± 2	184 ± 26	169 ± 30	93 ± 8	728 ± 136	562 ± 119
2	29 ± 3	76 ± 14	43 ± 11	90 ± 5	263 ± 28	182 ± 43
4	32 ± 4	47 ± 21	45 ± 23	83 ± 7	206 ± 46	142 ± 48
8	30 ± 2	40 ± 20	33 ± 20	85 ± 6	190 ± 30	120 ± 32

TABLE 12.

Therapeutic effect on cirrhosis (Histopathological examination)

Period of therapy (week)	Control	Positive control					Therapeutic group				
0	-----	++	+++	++	+	++					
1		++	+++	+++	++	++	+	+++	++	++	++
2		+	++	+++	+	++	+	+	±	±	++
4		+++	++	++	+	+++	±	±	+	+	±

It was considered that all the animals had reached the stage of cirrhosis after carbon tetrachloride was administered for a period of fourteen weeks. As shown in Table 11, the compound rapidly normalized plasma transaminase activity, both GPT and GOT.

Table 12 illustrated the therapeutic effect on cirrhosis. In the positive control group a moderate to high degree of cirrhosis including fibrosis of liver, appearance of pseudo lobulus and its cohesion was observed over a period of 8 weeks after the final administration of carbon tetrachloride. On the contrary, in the therapeutic group the cohesion of pseudo lobulus and the fibrosis were ameliorated after two weeks had passed, demonstrating the therapeutic effect of the compound on cirrhosis. Thus, the compound in this invention is useful as pharmaceutical for therapy chronic liver diseases and cirrhosis in humans and animals.

Example 16.

Cholagogic Action.

Formation and excretion of biliary salts in the bile outflow are the important metabolic functions of the liver. If the bile flow is damaged by some cause, cholestasis and several types of liver diseases accompanying jaundice will be induced.

Drugs as choleresis are used for patients to improve bile flow. In the present example, the effect of the compound of the invention on the quantities of bile and biliary salt is investigated.

Methods

The male rat (4 week-aged, SD-JCL strain) was anesthetized with sodium pentobarbital (37.5 mg/kg, intraperitoneal injection). The abdominal cavity was opened through a midline incision. The common bile duct was catheterized with polyethylene tube o.d. 0.8 mm. The catheter was brought to the outside through the abdominal incision wound prior to closing. Bile was collected from the catheter continuously with time intervals of one hour. The quantity of bile produced in one hour was determined by weighing and that of biliary salt was determined by enzymatic method (Ikagaku Jikkenho Koza 1B, Biological Constituents II, edited by Tamio YAMAKAWA). The amount of biliary salts was represented as cholic acid equivalent.

In the representation of the results, the quantities of bile and biliary salts produced in one hour prior to the administration of drug were taken as 100. The compound of this invention was orally administered at the dose of 200 mg/kg. As reference, anethol trithion was used.

Results

TABLE 13.
Effect on bile flow

Time	Experiment 1		Experiment 2	
	Control	Compound 4 (200 mg/kg)	Control	Anethol trithion (200 mg/kg)
Before treatment				
1 hour	100	100	100	100
After treatment				
1 hour	100 ± 2.4	115 ± 4.5	104 ± 3.5	114 ± 15
2	104 ± 6.5	122 ± 7.6	101 ± 4.9	114 ± 15
3	92 ± 6.4	117 ± 7.1	93 ± 6.9	102 ± 11
4	88 ± 9.7	114 ± 8.2	84 ± 6.7	95 ± 7.7
5	85 ± 9.2	102 ± 9.2	76 ± 6.6	86 ± 6.6

TABLE 14.
Effect on the excretion of biliary salt

Time	Experiment 1		Experiment 2	
	Control	Compound 4 (200 mg/kg)	Control	Anethol trithion (200 mg/kg)
Before treatment				
1 hour	100	100	100	100
After treatment				
1 hour	89 ± 6.6	108 ± 15	94 ± 6.2	108 ± 12
2	92 ± 11	132 ± 25	89 ± 9.5	89 ± 11
3	66 ± 8.8	138 ± 27	77 ± 11	66 ± 3.8
4	54 ± 15	117 ± 27	56 ± 9.7	47 ± 3.0
5	46 ± 16	90 ± 21	52 ± 10	33 ± 4.0

As shown in Table 13, bile flow was elevated by the administration of the compound of this invention. Quantity of total excreted bile for 5 hours after administration of Compound 4 was 1.2 times greater than that in control. In the case of anethol trithion the quantity of total excreted bile was 1.1 times greater than that in control.

As shown in Table 14, the amount of excreted biliary salts was remarkably increased by the administration of the compound of this invention. Amount of total biliary salts excreted for 5 hours after administration of Compound 4 was 1.7 times greater than that in control. In the case of anethol trithion, however, no particular increase was observed in the excretion of biliary salt. In other words, the compound of this invention is particularly effective in promoting the excretion of biliary salt.

The compound of this invention is to be classified as drug for increasing the excretion of biliary salts, namely cholanertica, and their effectiveness to the diseases of human or animal liver or bile duct can readily be expected from the hitherto reported findings concerning the behavior of anethol trithion. As above, the compound of this invention can stimulate the metabolic function of the liver and therefore is useful as pharmaceutical for controlling liver diseases caused by cholestasis.

Example 17.

Effect on Concentration of Ethyl alcohol in the Blood.

Methods

The test compounds were dissolved or suspended in olive oil and administered orally at the dose of 250 mg/kg to the male mouse (dd-strain). After 6 hours, 1,000 mg/kg of ethyl alcohol was given orally. Blood was taken in a capillary from caudal vein at the time indicated in the results. The plasma was obtained by centrifugation. The concentration of ethyl alcohol in the plasma was measured by FID type gas liquid chromatography. Five mice were used for each group.

Results

TABLE 15.

Effect on concentration of ethyl alcohol in the blood

Time (min.)	Concentration of ethyl alcohol in plasma (ppm)		
	Control	Compound pre-treated	
		Compound 4	Compound 7
0	27 ± 17	25 ± 12	30 ± 14
5	875 ± 203	582 ± 143	830 ± 180
15	810 ± 191	468 ± 30	799 ± 154
30	690 ± 214	220 ± 87	448 ± 208
60	167 ± 76	29 ± 17	158 ± 113
120	5 ± 1	not detected	12 ± 2

The concentration of ethyl alcohol in the plasma of the present compound-treated mice was lower than that of control. This tendency was particularly marked at the stages of 30, 60 and 120 minutes after administration of ethyl alcohol, and thus it was understood that the amount of ethyl alcohol in the treated mice decreased quickly. Further, the present compound-treated mice were obviously quicker in recovery of intoxicated state, when observed visually. This

Accordingly, the compounds of this invention are useful as pharmaceutical for lowering the concentration of alcohol in the blood.

Example 18.

The test compounds were dissolved in olive oil and administered orally at the dose of 250 mg/kg to the male mouse (dd-strain). After 6 hours, 4.0 g/kg of glucose was orally administered. The same amount of glucose was given to the control animals. 0.02 ml of blood was taken from caudal vein of the mice at 30, 60, 90 and 120 min. after glucose administration. Blood sugar was measured by the procedure of Somogyi-Nelson. Number of animals used was 5 to 6 mice for each treatment.

TABLE 16.

Effect on concentration of glucose in the blood

Time (min.)	Blood sugar (mg/dl)		
	Control	Test compound pre-treatment	
		Compound 2	Compound 4
0	145 ± 11.8 (100%)	136 ± 20.4 (100%)	123 ± 10.9 (100%)
30	311 ± 21.9 (214%)	295 ± 22.3 (217%)	282 ± 25.2 (229%)
60	290 ± 14.6 (200%)	251 ± 19.6 (185%)	236 ± 19.7 (192%)
90	263 ± 13.1 (181%)	202 ± 12.3 (149%)	159 ± 18.3 (129%)
120	251 ± 13.5 (173%)	158 ± 15.5 (116%)	138 ± 14.4 (112%)

The blood sugar values of the each group showed peaks after 30 minutes, and no substantial difference was seen in the peak values. Thereafter, however, obvious difference was observed in the recovery of blood sugar value, and the present compound-treated groups were quicker in recovery. This indicates that by administration of the present compounds, glucose metabolic function of liver was stimulated.

Accordingly, the compounds of this invention are useful as pharmaceutical for lowering abnormally elevated blood glucose concentration of humans and animals.

WHAT WE CLAIM IS:—

1. A pharmaceutical composition for controlling liver diseases, comprising (a) an effective amount of a dithiol derivative represented by the following general formula (I):



wherein R¹ and R², which may be same or different, represent individually a C₁—C₄ alkyl group, and (b) a pharmaceutically acceptable diluent.



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indicates that alcohol metabolic function of liver were stimulated by administration of the present compounds.

Accordingly, the compounds of this invention are useful as pharmaceutical for lowering the concentration of alcohol in the blood.

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Example 18.
Effect on glucose metabolism.

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Methods

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The test compounds were dissolved in olive oil and administered orally at the dose of 250 mg/kg to the male mouse (dd-strain). After 6 hours, 4.0 g/kg of glucose was orally administered. The same amount of glucose was given to the control animals. 0.02 ml of blood was taken from caudal vein of the mice at 30, 60, 90 and 120 min. after glucose administration. Blood sugar was measured by the procedure of Somogyi-Nelson. Number of animals used was 5 to 6 mice for each treatment.

10

Results

TABLE 16.

Effect on concentration of glucose in the blood

Time (min.)	Blood sugar (mg/dl)		
	Control	Test compound pre-treatment	
		Compound 2	Compound 4
0	145 ± 11.8 (100%)	136 ± 20.4 (100%)	123 ± 10.9 (100%)
30	311 ± 21.9 (214%)	295 ± 22.3 (217%)	282 ± 25.2 (229%)
60	290 ± 14.6 (200%)	251 ± 19.6 (185%)	236 ± 19.7 (192%)
90	263 ± 13.1 (181%)	202 ± 12.3 (149%)	159 ± 18.3 (129%)
120	251 ± 13.5 (173%)	158 ± 15.5 (116%)	138 ± 14.4 (112%)

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The blood sugar values of the each group showed peaks after 30 minutes, and no substantial difference was seen in the peak values. Thereafter, however, obvious difference was observed in the recovery of blood sugar value, and the present compound-treated groups were quicker in recovery. This indicates that by administration of the present compounds, glucose metabolic function of liver was stimulated.

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Accordingly, the compounds of this invention are useful as pharmaceutical for lowering abnormally elevated blood glucose concentration of humans and animals.

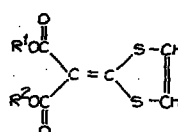
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WHAT WE CLAIM IS:—

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1. A pharmaceutical composition for controlling liver diseases, comprising (a) an effective amount of a dithiol derivative represented by the following general formula (I):

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(I)

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wherein R¹ and R², which may be same or different, represent individually a C₁—C₄ alkyl group, and (b) a pharmaceutically acceptable diluent.

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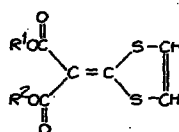
2. A pharmaceutical composition according to claim 1, wherein said dithiol derivative is diisopropyl 1,3-dithiol-2-ylidene malonate.

3. A pharmaceutical composition according to claim 1 or 2 which is in a form suitable for oral or parenteral administration.

5 4. A pharmaceutical composition according to claim 3 which is in aseptic form or in the form of an isotonic solution.

10 5. A pharmaceutical composition according to claim 4 which is in the form of a powder, granule, tablet, pill, sugar-coated tablet, capsule, ampoule or suppository.

6. A process for controlling liver diseases of non-human animals which comprises administering to the animals, orally or parenterally, an effective amount of dithiol derivative represented by the general formula (I) as an active ingredient in the form of a mixture with a pharmaceutically acceptable diluent:



(I)

15 wherein R¹ and R², which may be same or different, represent individually a C₁—C₄ alkyl group.

7. A process according to claim 7 which comprises administering said dithiol derivative orally at the dose of 1 to 100 mg per one kilogramme of animal body weight per day.

20 8. A process according to claim 7 which comprises administering said dithiol derivative orally at the dose of 1 to 100 mg per one kilogramme of animal body weight per day.

25 9. A process according to claim 6 which comprises administering said dithiol derivative parenterally at the dose of 0.01 to 250 mg per one kilogramme of animal body weight per day.

10. A process according to claim 9 which comprises administering said dithiol derivative parenterally at the dose of 0.1 to 25 mg per one kilogramme of animal body weight per day.

30 11. A pharmaceutical composition for controlling liver diseases according to claim 1, substantially as hereinbefore described in any of the Examples.

12. A process for controlling liver diseases in non human animals according to claim 6, substantially as hereinbefore described with reference to any of the Examples.

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