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(72) Inventeur/Inventor:
GILAT, TUVIA, IL
(73) Propriétaire/Owner:
GALMED INTERNATIONAL LIMITED, MT
(74) Agent: BORDEN LADNER GERVAIS LLP

(54) Titre : UTILISATION DE CONJUGUES D'ACIDE BILIAIRE OU D'ACIDE GRAS DE SEL BILIAIRE
(54) Title: USE OF BILE ACID OR BILE SALT FATTY ACID CONJUGATES

(57) **Abrégé/Abstract:**

The present invention relates to the use of a bile acid or bile salt fatty acid conjugate of general formula II W - X - G in which G is a bile acid or bile salt radical, which, if desired, is conjugated in position 24 with a suitable amino acid, W stands for one or two fatty acid radicals having 14-22 carbon atoms and X stands for a suitable bonding member or for a direct C=C bond between said bile acid or bile salt radical and the fatty acid(s) or of a pharmaceutical composition comprising same for the reduction of Cholesterol in blood, for the treatment of Fatty Liver, Hyperglycemia and Diabetes.



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(71) Applicant (*for all designated States except US*):
GALMED INTERNATIONAL LIMITED [MT/MT];
35 Mannarino Road, BKR 08 B'Kara (MT).

(72) Inventor; and

(75) Inventor/Applicant (*for US only*): **GILAT, Tuvia** [IL/IL];
Nehardea St. 10, 64235 Tel-Aviv (IL).

(74) Agent: **DR. YITZHAK HESS & PARTNERS**; P.O. Box
6451, 61063 Tel-Aviv (IL).

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(54) Title: USE OF BILE ACID OR BILE SALT FATTY ACID CONJUGATES

(57) Abstract: The present invention relates to the use of a bile acid or bile salt fatty acid conjugate of general formula II W - X - G in which G is a bile acid or bile salt radical, which, if desired, is conjugated in position 24 with a suitable amino acid, W stands for one or two fatty acid radicals having 14-22 carbon atoms and X stands for a suitable bonding member or for a direct C=C bond between said bile acid or bile salt radical and the fatty acid(s) or of a pharmaceutical composition comprising same for the reduction of Cholesterol in blood, for the treatment of Fatty Liver, Hyperglycemia and Diabetes.



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USE OF BILE ACID OR BILE SALT FATTY ACID CONJUGATES

The present invention relates to new uses of certain bile acid or bile salt fatty acid conjugates.

From International Patent Application No. WO 99/52932 there are known bile acid or bile salt fatty acid conjugates [BAFAC I] of general formula I



in which G is a bile acid or bile salt radical, which if desired, is conjugated in position 24 with a suitable amino acid, W stands for one or two fatty acid radicals having 18-22 carbon atoms and X stands for a NH bond between said bile acid or bile salt radical and the fatty acid(s).

From said specification there is known the use of the compounds of general formula I and of pharmaceutical compositions comprising same for dissolving cholesterol gallstones in bile, preventing the occurrence or recurrence of said gallstones, and in reducing or preventing arteriosclerosis. There are also known methods for the treatment of said diseases.

It has now surprisingly been found that BAFACS and pharmaceutical compositions comprising same in which W stands for one or two fatty acid radicals having 14 – 22 carbon atoms and X stands for a suitable bonding member or for a direct C=C bond between said bile acid or bile salt radical and the fatty acid[s] (being compounds of general formula II; hereinafter called BAFACS II) and pharmaceutical compositions comprising same, have additional uses, namely they can be used:

- a. for the reduction of cholesterol concentration in blood;
- b. for the treatment of fatty liver; and
- c. for the treatment of hyperglycemia and diabetes.

Said BAFACS and pharmaceutical compositions comprising same can also be used in the treatment of said diseases.

The bond has to be a solid bond that is not substantially deconjugated by intestinal and/or bacterial enzymes during the process of absorption. An ester bond is thus not suitable since it is easily deconjugated. The bond stands in particular for NH but may also stand for other suitable bonding members, e.g. S, P, O-ether, etc.

An exemplary embodiment of the compound described herein of general formula II is one in which the fatty acid is behenyllic acid, arachidylic acid or stearic acid.

The bond can be in the alpha or beta configuration and can be attached in various positions of the bile acid molecule, positions 3, 6, 7, 12 and 24 being preferred.

The diseases concerned, the history of the treatment thereof and the new use for the treatment thereof are explained hereinafter:

A. Reduction of cholesterol concentration in blood

Hypercholesterolemia is deleterious to health. It is a causative factor in several major disease processes such as ischemic heart disease, myocardial infarction, peripheral vascular disease, possibly stroke, etc. Reduction of cholesterol concentration in blood is beneficial or preventive in some of said diseases. Current medical treatment of hypercholesterolemia is aimed at reducing endogenous cholesterol synthesis in the liver. The statins used for this purpose inhibit the enzyme HMG CoA Reductase. However, a major portion of body cholesterol originates from dietary cholesterol. Diet restrictions are notoriously ineffective. Ion exchange resins have also been used to bind bile acids (products of cholesterol catabolism) and sequester them in the intestinal lumen leading to their fecal excretion. They have some effect on blood cholesterol but also important side effects which limit their use.

It has now been shown that BAFACs II reduce diet induced hypercholesterolemia in several animal species even as the animals continue to consume the high cholesterol, high fat diet.

B. Treatment of Fatty Liver

Fatty liver is one of the most common liver diseases today. It is due to excessive accumulation of fat in the liver. It is demonstrated histologically by the presence of variable amounts of micro and/or macro vesicular fat droplets in the liver tissue. Fatty liver can be caused by drugs, chemicals, diseases, bacteria, etc. but the main cause is excessive dietary intake leading to (mainly truncal) obesity.

Due to the increasing prevalence of overweight in affluent societies the prevalence of fatty liver is rising. Fatty liver may progress to steatohepatitis and cirrhosis with the attendant morbidity and mortality.

The best treatment for diet induced fatty liver is sustained weight loss. As is, however, well known this is rarely achieved.

It has now been discovered that BAFACs II can reduce and prevent fatty

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liver. This has also been demonstrated during continuation of the excessive dietary intake in several animal species.

Thus, according to an aspect of the invention, there is provided a use of a bile acid or bile salt fatty acid conjugate (BAFAC) of general formula II



in which G is a bile acid or bile salt radical, which, if desired, is conjugated in position 24 with a suitable amino acid, W stands for one or two fatty acid radicals having 14-22 carbon atoms and X stands for a suitable bonding member or for a direct C=C bond between said bile acid or bile salt radical and the fatty acid(s), with the proviso that said bond is not an ester bond, wherein said suitable bonding member is NH, O, P or S, or a pharmaceutical composition comprising same for the treatment of Fatty Liver.

A further aspect of the invention provides a use of a bile acid or bile salt fatty acid conjugate (BAFAC) of general formula II



in which G is a bile acid or bile salt radical, which, if desired, is conjugated in position 24 with a suitable amino acid, W stands for one or two fatty acid radicals having 14-22 carbon atoms and X stands for a suitable bonding member or for a direct C=C bond between said bile acid or bile salt radical and the fatty acid(s), with the proviso that said bond is not an ester bond, wherein said suitable bonding member is NH, O, P or S, or a pharmaceutical composition comprising same for preparation of a medicament for the treatment of Fatty Liver.

C. Treatment of Hyperglycemia and Diabetes

Diabetes mellitus is a disorder of carbohydrate (glucose) metabolism. It is schematically divided into type 1, insulin dependent diabetes mellitus (IDDM) characterized by insulin deficiency and type 2, not insulin dependent diabetes mellitus (NIDDM) in which there is mainly insulin resistance. NIDDM is often associated with obesity and/or fatty liver. There are also other causes of hyperglycemia. Treatment of diabetes mellitus involves diet, insulin injections and/or oral hypoglycemic drugs. The aim of therapy is to normalize blood glucose levels. Diabetes, particularly poorly controlled diabetes leads to severe complications.

It has now been discovered that BAFACs reduce and normalize blood glucose levels in animal models of IDDM and NIDDM.

The present invention will now be described with reference to the following Examples and Figs. without being limited to them.

In said Examples the "regular diet" used had the following composition:

Carbohydrates 50%, Protein 21%, Fat 4% with mineral, fiber, and vitamin supplements (manufactured by Koffolk, Petach Tiqwa, Israel).

In the Figures,

Figs. 1A, 1B and 1C show fasting blood glucose concentrations in rodents on different regimens with or without BAFAC (150 mg/kg/day), i.e.:

Fig. 1A for Rats (4w)

Fig. 1B for Hamsters (3w)

Fig. 1C for Mice (16d)

and

Fig. 2 shows the lipid/protein ratio in the liver of inbred C57J/L mice fed a high fat diet +/- BAFAC (150 mg/kg/day) for five weeks.

Example 1

1. Hamsters Male

Golden Syrian Hamsters (Anilab, Rehovot, Israel) 4-6 weeks old weighing 90-110 gm were used. They were fed a lithogenic diet(No.1) consisting of their regular diet supplemented with (w/w) cholesterol 1%, Palmitic Acid 1.2%, Corn Oil

2% (modified from. Ayyad et al., Lipids, 1992;27:993-998) for 10 weeks. Half of the animals were given in addition 3β -arachidylamido- $7\alpha,12\alpha$ - dihydroxy- 5β -cholan-24-oic acid at a dose of 150mg/kg/day suspended in saline, by intragastric gavage. The control animals were similarly given an equal volume of saline. After ten weeks the animals were anesthetized, heart blood was drawn for analysis. The liver and other organs were removed. Serum cholesterol levels were determined by autoanalyzer.

2. Mice

Inbred C57J/L male mice (Jackson Lab., Maine, USA) 4-6 weeks old weighing 20-25 gm were used. They were given their regular diet supplemented with (%w/w) Butter fat 15, Cholesterol 1, Cholic Acid 0.5, Corn Oil 2 (modified from Khanuja et al., Proc.Natnl.Acad.Sci.USA 1995;92:7729-33) for 4-8 weeks.(Lithogenic diet #2) Half of the group were given 3β -arachidylamido- $7\alpha,12\alpha$ - dihydroxy- 5β - cholan-24-oic acid at a dose of 150 mg/kg/day suspended in saline by intragastric gavage. The other half were similarly given an equal volume of saline. After 4-8 weeks the animals were anesthetized, heart blood was drawn for analysis. The liver and other organs were removed. Serum cholesterol levels were determined by autoanalyzer.

Another group of C57 J/L male inbred mice (Jackson Laboratories, Maine,USA) 4-6 weeks old weighing 20-25 gm. were given a lithogenic diet (No.2) for 8 weeks and then a regular diet for another 8 weeks. During the 8 weeks of the regular diet part of the animals were given 3β -arachidylamido- $7\alpha,12\alpha$ - dihydroxy- 5β -cholan-24-oic acid by intragastric gavage at a dose of 150 mg/kg/day. After a total of 16 weeks the animals were anesthetized, heart blood was drawn for analysis. Liver and other organs were removed. Serum cholesterol levels were analyzed as above. A third group of animals were given a regular diet throughout the 16 weeks and then similarly analyzed.

The number of animals in each group and the results are given in the following Table 1

Table 1: Serum Cholesterol (mg%) of Test Animals (mean +StD)

Animals	Diet	Duration (W)	n	Diet Only	n	Diet +BAFAC II
Mice, inbred C57 J/L	Lithogenic 2	4	5	270 (21.7)	5	143 (19.8)
"	"	6	6	274 (4.1)	7	125 (10.8)
"	"	8	5	264 (5.8)	5	139 (0.7)
Hamsters, Golden Syrian	Lithogenic 1	10	5	257 (32.7)	5	202 (59.6)
Mice, inbred C57 J/L	Lithogenic 2 then regular	8 } 16 8	6	101 (10.1)	7	65 (6.8)
"	Regular only	16	4	81 (5.5)		

The data given in Table 1 show that the BAFACs II markedly reduce blood cholesterol levels in numerous groups of mice and hamsters.

Example 2Histologic Examination Method

The presence of fatty liver was evaluated by a pathologist unaware of the treatments, scoring coded slides in a blinded manner.

The scoring system was as follows:

0 – No fatty liver

1 – Minimal fatty liver <5 % of liver surface affected

2 – Mild fatty liver 5- <25 % of liver surface affected

3 - Marked fatty liver 25-50% of liver surface affected

4- Severe fatty liver >50% of liver surface affected

1. Hamsters

Golden Syrian Male Hamsters (Anilab, Rehovot, Israel) 4-6 years old weighing 90-110 gm were used. They were fed a lithogenic diet (No1). Half of the animals were given in addition 150 mg/kg/day of 3 β -arachidylamido-7 α ,12 α - dihydroxy-5 β -cholan-24-oic acid. The compound was given suspended in saline, by intragastric gavage. Control animals were similarly given saline only. After 10 weeks the animals were anesthetized and sacrificed. The liver and other organs were removed and placed in formaline for histologic examination.

2. MICE

C57 male inbred mice (Jackson Lab., Maine, USA) 4-6 weeks old weighing 20-25 gm were used. They were fed a "Western" diet (George et.al.Circulation 2000,102:1822-27) containing cholesterol 1.5 gm/kg and Fat 42%, Carbohydrates 43%, and Protein 15% (as % of calories). Half were given in addition 150 mg/kg/day of 3 β -arachidylamido-7 α ,12 α -dihydroxy-5 β -cholan-24-oic acid suspended in saline by intragastric gavage. The other half were similarly given saline only. After 4 weeks the animals were anesthetized and sacrificed. Liver and other organs were removed and placed in formaline prior to histologic examination

The number of animals in each group and the histologic results are given in the following Table 2.

Table 2: Fatty Liver Scores of Test Animals

Animals	Diet	Duration (W)	n	Control (scores)						n	+BAFAC (scores)					
				0	1	2	3	4	mean		0	1	2	3	4	mean
Hamsters, Golden Syrian	Lithogenic 1	10	7					7	4a	7	6		1			0.28
Mice C57 J/L Inbred	Western	4	10	1	3	6			1.5b	14	12	1			1	0.35

a-Microvesicular fat

b-Mixed (micro and macrovesicular) fat

The data in said Table 2 show that BAFACS reduce and/or prevent fatty liver.

Example 3

The effects of BAFACs on fasting blood glucose were tested in 3 animal species: Hamsters, Rats and Mice. The animals were kept on regular or high fat diets (as specified). In each group half of the animals received a daily BAFAC dose (150mg/kg) dispersed in 0.5 ml of saline by gavage. The other half received similarly, by gavage, an equal volume of saline only. All animals were kept on a 12 hr day/night cycle at 22°C throughout the experiment. They had access to water ad libidum

1. Hamsters

Golden Syrian male hamsters 4 weeks old (90-100gm) were fed a fatty liver diet consisting of cholesterol (1%), palmitic acid (1.2%), butter (6%), lard (10%), and corn oil (2%) added (w/w) to their regular diet. Blood glucose was measured at sacrifice in the fasting state after 3 weeks of the trial. There were 5 control and 5 test animals.

2. Rats

Wistar male rats 4 weeks old (100-120gm) were fed a regular chow diet or a high fat diet enriched with lard (10%), cholesterol (2.5%) and cholic acid (0.5%) (w/w). Blood and organ samples were obtained after sacrifice (in the fasting state) after 4 weeks of the trial. Half of the animals in each group were given BAFAC 150mg/kg; the others were given an equal volume of saline. There were 7 rats in the control and test groups on a regular diet and 6 rats in each group given a high fat diet.

The hamsters and rats on the high fat diets developed fatty liver (confirmed by chemical measurement of liver fat), a high fasting blood glucose, and represented models of NIDDM.

3. Mice

ICR male mice, 4 weeks old (approx. 20gm) were fed a regular diet. Streptozotocin 200 mg/kg was injected i.p. on the first day of the experiment. (This induced damage to the islet cells of the pancreas and simulated IDDM). Fasting blood glucose was obtained from sacrificed animals after 16 days of the trial. There were 3 groups of 7 animals each. Controls – regular diet only. Streptozotocin (Stz) treated animals receiving saline only by gavage and streptozocin treated animals receiving 150mg/kg/day of BAFAC by gavage. The BAFAC used in all these hyperglycemia studies was a conjugate of arachidic and cholic acid (at position 3) using an amide bond (C-20 BAFAC, Aramchol).

The results of the 3 studies are shown in Figs. 1A, 1B and 1C. (RD-Regular diet, FLD – Fatty Liver Diet, Stz = Streptozotocin.)

It can be seen that BAFAC supplementation markedly reduced fasting blood glucose in all test animals. Blood glucose was reduced in animals with fatty liver (Hamsters and Rats) – representing NIDDM as well as in the streptozotozin treated mice representing IDDM. BAFACs had no effect on blood glucose in rats on a normal diet (with no hyperglycemia or fatty liver)

Example 4

Inbred C57J/L mice, 4 weeks old and weighing approx. 20gm (Jackson Lab. Maine, USA) were used. They were fed for 5 weeks a high fat lithogenic diet consisting of: Cholesterol 1%, Cholic Acid 0.5%, lard 10%, butter 6%, palmitic acid 1.2% and corn oil 2% added (w/w) to their regular diet. In addition to the diet the animals were given daily, by gavage, either a BAFAC(150mg/Kg) suspended in saline or an equal volume of saline only. The BAFACs were conjugates of cholic acid (at position 3) with either Palmitic Acid (C-16) or Arachidic Acid (C-20) or Behenic Acid (C-22) using an amide bond. The controls (n=5) received saline, the test animals received either the C-16 conjugate (n=5), the C-20 conjugate (n=5) or the C-22 conjugate (n=3). After 5 weeks, following an overnight fast, the animals were anesthetized using ketamine, the liver was removed, and the animals were sacrificed by ketamine overdose. A 0.5gm sample of liver was homogenized in 5ml of buffered saline. Liver lipids were extracted from the homogenate by the Folch method using chloroform: methanol (2:1, vol/vol). Half a ml of the homogenate were extracted in 5ml of the Folch solution. After evaporation of the solvent the lipids were weighed and reevaporated several times until constant weight was obtained. The proteins were quantified in another aliquot of the homogenate according to the method of Bradford.(Bradford M.M., Annal. Biochem. 1976,72:248)

The lipid protein ratio (mg/mg) was calculated. The results for each group are given as means (+St. Dev.) The group values were:

Controls 7.9+/-2.32 , C-16 BAFAC 2.25 +/-1.20 , C-20 BAFAC 1.44+/-1.18, and C-22 BAFAC 3.00+/-1.08 . The results are illustrated in Fig 2.

The data show that the effects claimed are exercised by several conjugates, some of them almost as potent as the C-20 conjugate used in examples 1, 2 & 3.

CLAIMS

1. Use of a bile acid or bile salt fatty acid conjugate (BAFAC) of general formula II



in which G is a bile acid or bile salt radical, which, if desired, is conjugated in position 24 with a suitable amino acid, W stands for one or two fatty acid radicals having 14-22 carbon atoms and X stands for a suitable bonding member or for a direct C=C bond between said bile acid or bile salt radical and the fatty acid(s), with the proviso that said bond is not an ester bond, wherein said suitable bonding member is NH, O, P or S, or a pharmaceutical composition comprising same for the treatment of Fatty Liver.

2. The use according to claim 1, wherein in general formula II the bonding member is NH.
3. The use according to claim 1, wherein in general formula II the fatty acid is behenylic acid, arachidyllic acid or stearic acid.
4. The use according to claim 1, wherein general formula II is 3 β -arachidylamido-7 α ,12 α -dihidroxy-5 β -cholan-24-oic acid.

5. Use of a bile acid or bile salt fatty acid conjugate (BAFAC) of general formula II



in which G is a bile acid or bile salt radical, which, if desired, is conjugated in position 24 with a suitable amino acid, W stands for one or two fatty acid radicals having 14-22 carbon atoms and X stands for a suitable bonding member or for a direct C=C bond between said bile acid or bile salt radical and the fatty acid(s), with the proviso that said bond is not an ester bond, wherein said suitable bonding member is NH, O, P or S, or a pharmaceutical composition comprising same for preparation of a medicament for the treatment of Fatty Liver.

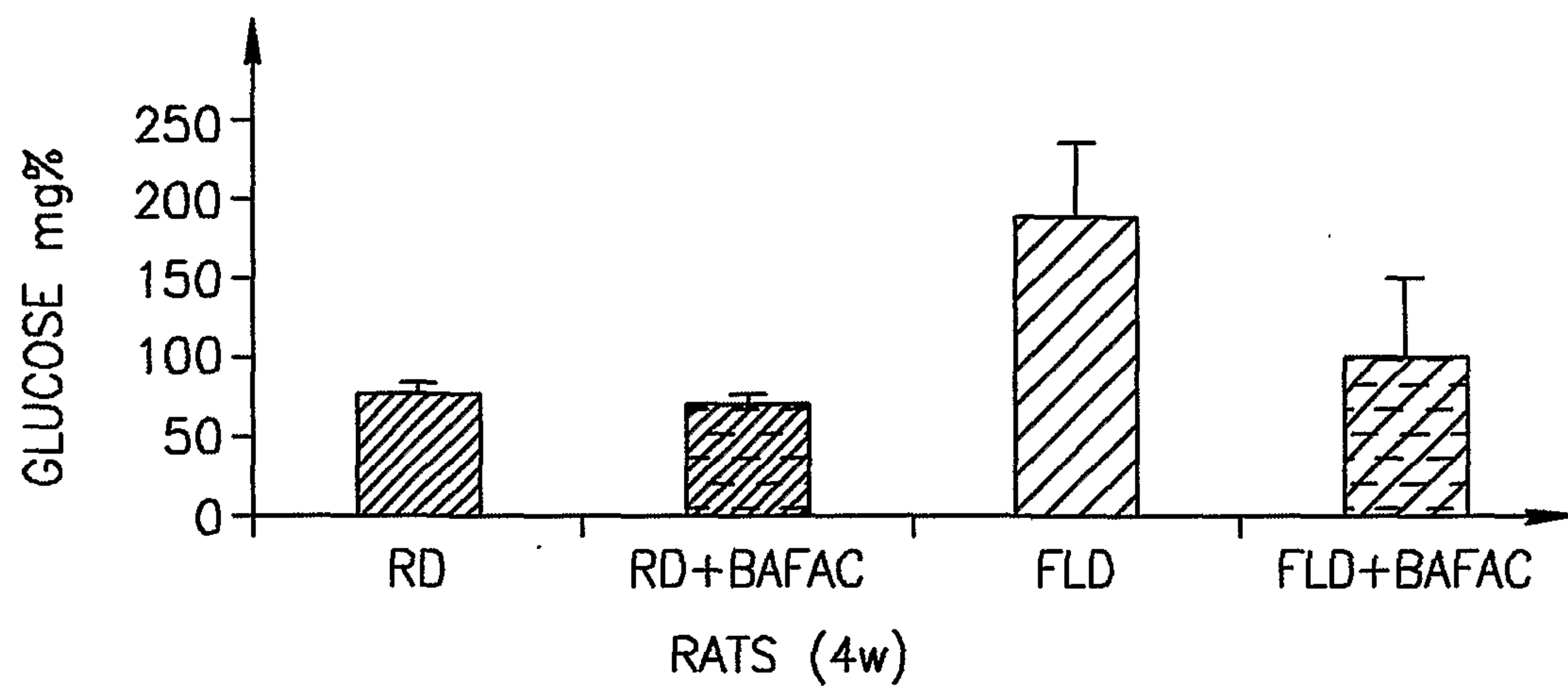
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FIG.1A

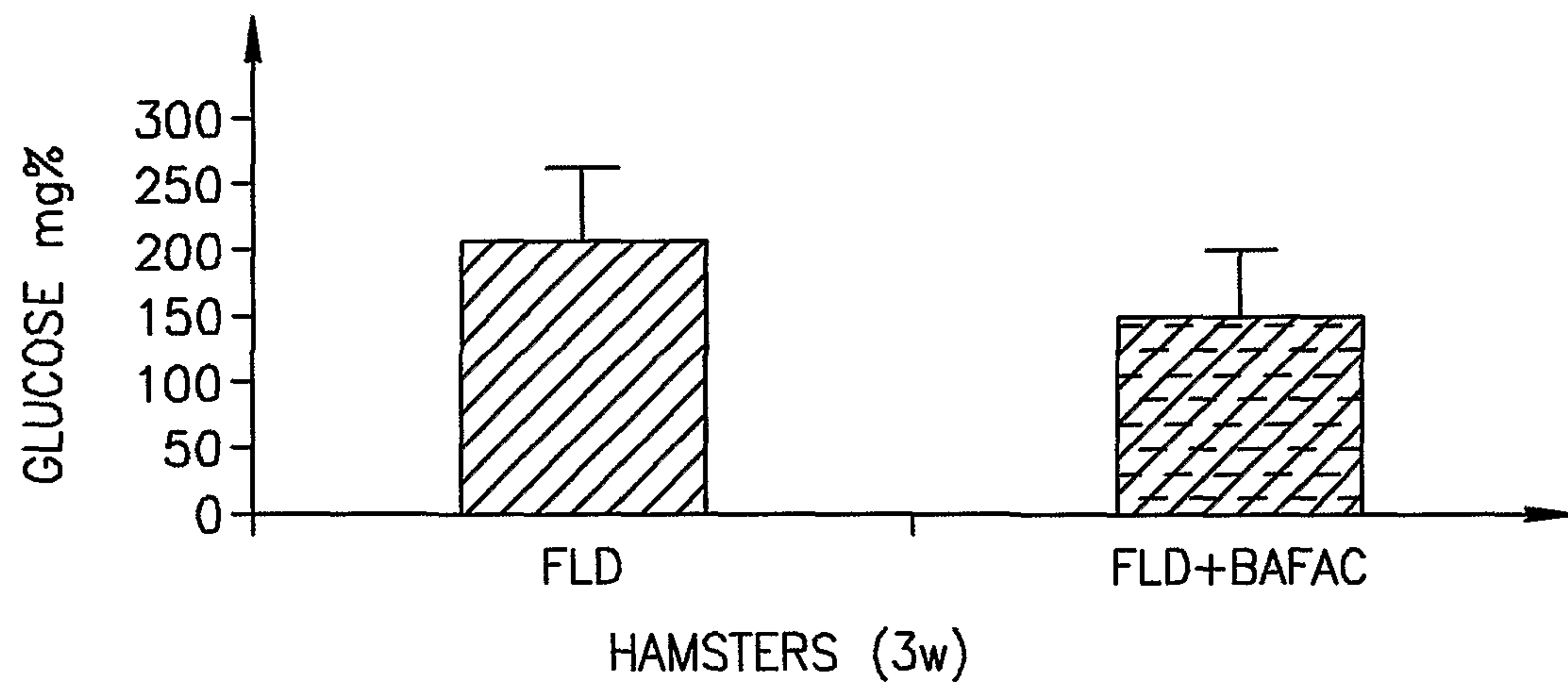


FIG.1B

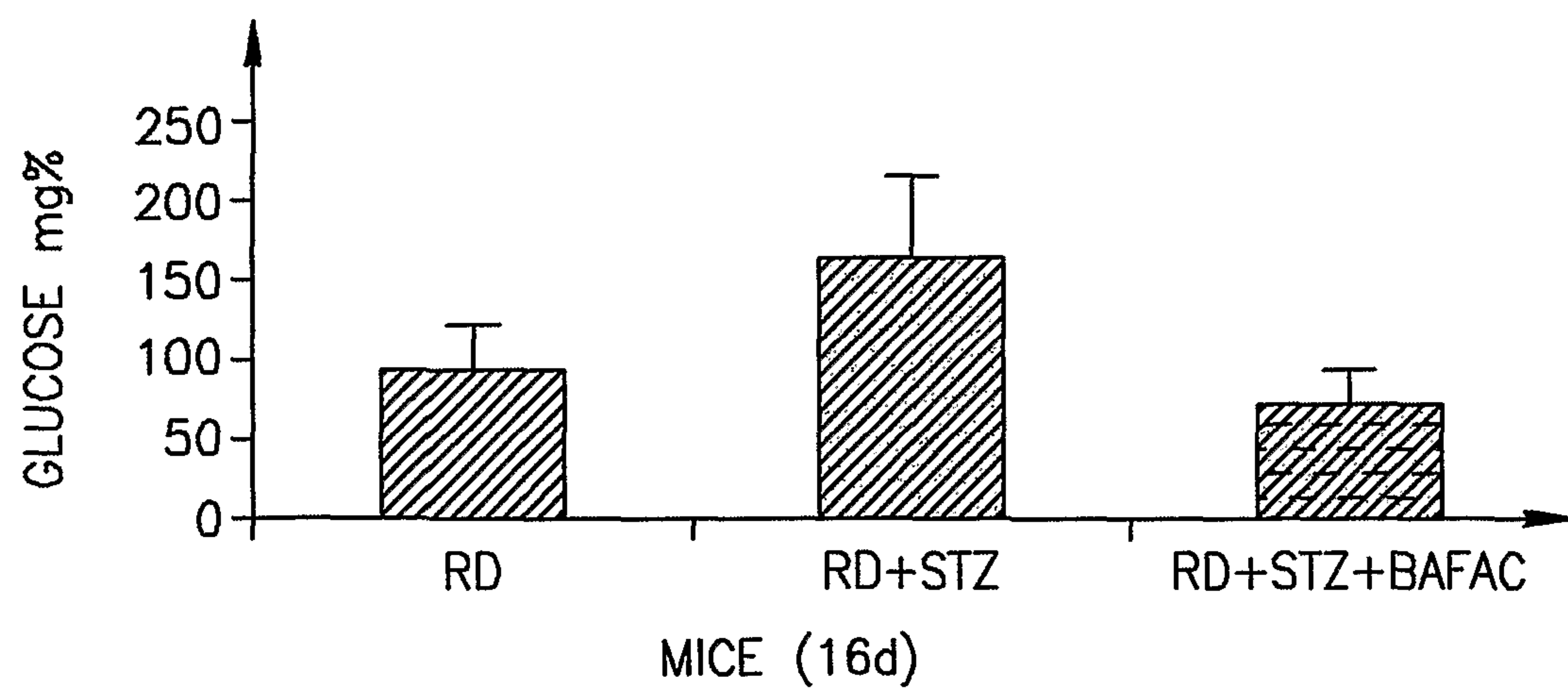


FIG.1C

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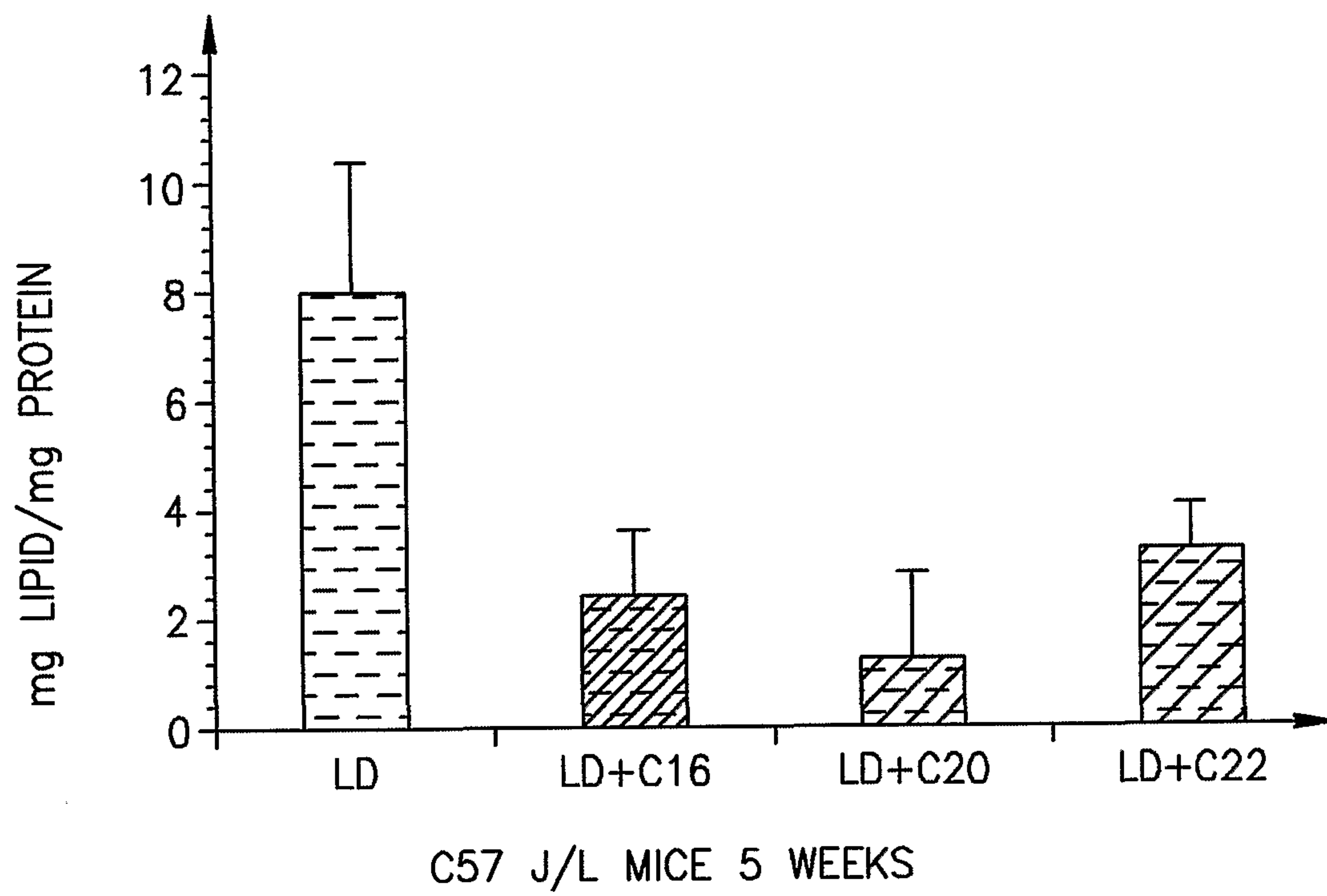


FIG.2